GENERALIZATION OF PREDATOR RECOGNITION IN FATHEAD MINNOWS: IMPLICATIONS FOR RESPONSES TO INTRODUCED AND HYBRID PREDATORS

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Masters of Science
in the Department of Biology
University of Saskatchewan
Saskatoon

By
Jessica Lynn Popp
Winter 2016

Permission to Use

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Biology
112 Science Place, University of Saskatchewan
Saskatoon, Saskatchewan
S7N 5E2, Canada

Abstract

To survive, prey must recognize predators and appropriately respond to the associated risk. Some prey must first gain this information through experience. Naïve fathead minnows (*Pimephales promelas*) learn to recognize a predator after a single learning event, and subsequently generalize their antipredator responses to odours of novel, closely-related predators. This phenomenon known as 'generalization of predator recognition' has been observed in fishes, amphibians, reptiles, and mammals via generalization of both olfactory and visual cues. The extent of generalized predator recognition has been a topic of interest for researchers over the past 10 years, providing a wider expanse of knowledge that has begun to uncover the circumstances facilitating generalization and those that do not. The overall purpose of my research was to explore one aspect that has received minimal attention—the ability for prey to generalize response to frequently stocked hybrid predators. I first investigated the capacity for minnows to generalize to hybrid tiger trout odour after alarm-cue learning of either one or both of the parental species' odours. My results in Chapter 2 showed that regardless of conditioning odours, minnows were able to generalize their antipredator response from the known parental species to the novel hybrid predator and to the novel confamiliar parental species. Following the results of Chapter 2, I was interested in understanding whether knowledge of parental species was important for hybrid tiger trout recognition or if knowledge of any trout would be conducive to facilitating generalized predator recognition of tiger trout odour. Chapter 3 was designed to investigate this question. Results from this experiment reinforced that knowledge of one or both parental species would be necessary for adequate generalization of hybrid predator odours. Minnows conditioned to learn a confamiliar species to both parental trout did not respond to the odour of novel tiger trout. Results of both experiments were also analyzed to observe the intensity of antipredator response over the 8-min post-stimulus observation. These analyses indicated that prey may indeed exhibit graded antipredator responses based on the degree of phylogenetic similarity between the known and novel predators, evidence of which may be concealed when observing only the overall response to novel odours. With the increasing prevalence of ecological invasions and continued introduction of fish species into aquatic habitats, knowledge of how prey will respond to novel species may illuminate how susceptible populations and ecosystems may be. My results highlight the importance of accounting for lake and stream composition and the level of prey naïvety prior to introducing hybrid predators.

Acknowledgements

Move to Canada, they said. It'll be fun, they said…and boy, were they right! My time in Saskatoon has been nothing short of an adventure, and it would not have been possible without support from so many key people in my life.

I would first like to thank Dr. Maud Ferrari, for taking a chance on the nomadic American. Your guidance and support throughout my thesis were instrumental in opening my eyes to the wonderful world of fish. You always knew when to ask the deeper questions, tame down my larger-than-feasible experimental designs, and put on the pressure when I needed it the most. I especially appreciate your acceptance of my unrelenting determination to balance oh-so-many hats of involvement over these past few years. In all, it has made my experience a challenging and highly rewarding one.

I would like to thank my committee members, Dr. Jeff Lane and Dr. Doug Chivers, for their time and assistance throughout my thesis. Additional thank you to my external examiner, Dr. Jeff Sereda for your interest in my thesis and thoughtful questions surrounding my research's applicability. Thank you all for continuing to bring about new perspectives into my learning and thought processes.

I would like to express a mountain of gratitude to my office mates, lab mates, fellow graduate students, and roommates whose friendship and support have made this thesis attainable and enjoyable. Thank you to all those who helped me collect minnows in the wee hours of the morning, shared countless fires, BBQs, hikes, fishing and hunting trips, and listened to both my struggles and successes. You all helped to make Saskatoon a place to call home. Thank you to Janelle Sloychuk and the Cold Lake Fish Hatchery (AB) for measuring over 120 trout and preparing and donating the odour necessary for me to be able to scare my little minnows.

A special thank you to Adam Crane and Brandon Demuth—the two of you made me feel instantly welcome in our lab. Brandon, thank you for inadvertently providing me with my first introductory lessons on fish research (and patience with all of my novice questions) and welcoming me to many Friendsgivings (American and Canadian), Easters, football nights, and more. Adam, thank you for taking me under your wing and going above and beyond in your mentorship. You gave me to tools to be independent in the lab—guiding me through experimental designs in our study system, furthering my understanding of statistical analyses, welcoming an opportunity to collaborate, and most importantly, continually providing advice and support as a friend and a colleague. The two of you were invaluable throughout this process.

Finally, I would like to thank my family, for your unwavering support of my adventures across North America. To my sister, Amber—thank you for always listening and being the person I can turn to for anything in the world. Your support, advice, and love have driven me to follow my dreams (and always make sure I come home for the holidays). To my brother, Jason—thank you for always making me laugh…and cry. Your ability to lighten the mood has saved me in more than one occasion. To my father, Jeff—thank you for your love and encouragement over my years away. You taught me the value of hard work and perseverance, and always challenged me to be my best. To my mother, Melissa—thank you for always reminding me of the bigger picture

and supporting my crazy decisions—even when those decisions include us camping in freezing temperatures, having our car charged by a bison, and driving for hours without seeing a single person on Highway 4 into Canada. And last but not least, Harrison—thank you for taking this adventure through graduate school with me. I wouldn't have been able to accomplish all that I have without your unwavering encouragement, sound reasoning, and immense knowledge. Thank you for being my shoulder to lean on and providing me with many necessary breaks—especially the ones from watching fish in the lab to catching fish in the outdoors with you and Darwin.

Research funding for the work presented in this thesis was provided by the Natural Sciences and Engineering Research Council Discovery Grant to M.C.O. Ferrari. Personal funding was provided by the University of Saskatchewan (CGSR and Dept. of Biology), the Graduate Service Fellowship, and the Wolfe Family Scholarship. All the work reported herein followed UCACS protocols no. 20130079 and 20150059.

Table of Contents

List of Figures

Chapter 1: Introduction

1.1 Mechanisms for Predator Recognition

In order to survive, prey must successfully avoid becoming a predator's next meal. Although prey could incessantly flee from all potential predators and avoid dangerous habitats, it would be impossible—prey need to balance predator evasion along with other fundamental necessities in life (i.e. foraging, courting, reproducing, etc.) (Lima & Dill, 1990). To do so, prey must be able to recognize potential predators and appropriately respond to the associated risk. For some organisms, this recognition is an innate characteristic, exhibiting the ability to respond to predators during their first encounter with no individual history or experience (e.g. chinook salmon (*Oncorhynchus tshawytscha*), Berejikian, Tezaka, & LaRaeb, 2003; orange clownfish (*Amphiprion percula*), Dixson, Munday, & Jones, 2010; paradise fish (*Macropodus opercularis*), Gerlai, 1993). Innate recognition and response may pose a significant survival advantage if strong predation pressure exists for young age classes, if predation is highly predictable with a stable predator community, or if the probability of surviving an encounter is minimal (Ferrari, Gonzalo, Messier, & Chivers, 2007; Murray, Roth, & Wirsing, 2004; Wisenden, 2003). As co-evolutionary history between predator and prey expands, innate recognition would also become increasingly beneficial to prey, minimizing the threat and necessary time associated with learning events (Ferrari et al., 2007; Kats & Ferrer, 2003).

Conversely, a diverse array of aquatic species lack the innate ability to recognize potential predators (e.g., invertebrates, Hazlett, 2003; Wisenden & Millard, 2001; fishes, Chivers & Smith, 1994b, 1998; Mathis & Smith, 1993; amphibians, Mirza, Ferrari, Kiesecker, & Chivers, 2006; Woody & Mathis, 1998). In environments with a diverse range of predators and low temporal and/or spatial predictability, for instance, it would be advantageous for prey to base their response on their most recent experiences (Ferrari et al., 2007; Kelley & Magurran, 2003). There exists an apparent trade-off of energy and time allocation between predator avoidance and other fitness-related behaviours; thus, prey would benefit from being able to assess the current level of threat, respond appropriately in various contexts, and subsequently maximize energy and

time devoted to other activities (Ferrari & Chivers, 2006; Ferrari, Trowell, Brown, & Chivers, 2005; Helfman, 1989; Lima & Bednekoff, 1999).

1.2 Learned Predator Recognition and Avoidance

Predator avoidance strategies that incorporate learning and behavioural plasticity are important for prey to maintain up-to-date local information, allowing for enhanced assessment of environmental risk at a given place in time (Ferrari & Chivers, 2006; Kats & Dill, 1998). Prey have exhibited the ability to associate specific levels of risk with a learned predator (Ferrari & Chivers, 2009; Ferrari et al., 2005; Hill & Weissburg, 2014; Lönnstedt & McCormick, 2011; Mathuru et al., 2012). As predation threats vary over a prey's lifetime, animals that adjust the intensity of their response based on the present level of threat would obtain the highest survival benefit, accurately assessing and responding to risk without wasting unnecessary energy (Helfman, 1989; Wisenden, 2003).

The use of experience via learning does not, however, come without its costs. Learning through experience has a higher associated risk than innate recognition, as prey must first survive an encounter with a predator before gaining applicable knowledge. In aquatic environments, using chemical cues may reduce the amount of risk associated with learning events. Although sometimes less reliable, chemical cues can be utilized at a greater, and probably safer, distance than visual cues allow (Brown, 2003; Ferrari, Wisenden, & Chivers, 2010), especially in low-light or murky environments (Kattenfeld & Abrahams, 1997).

Ostariophysan fishes (i.e. minnows, suckers, catfishes, carp, etc.) possess specialized skin cells, or club cells, in the epidermis that generate a damage-released chemical alarm substance (herein referred to as 'alarm cue') (Chivers & Smith, 1998; Wisenden, 2000; Wisenden, Vollbrecht, & Brown, 2004). When attacked or captured by a predator, damage to the epidermis releases these alarm cues into the water. Upon detection of this cue, other conspecifics will innately recognize the cue and exhibit significant antipredator behaviours, such as freezing, dashing, increasing shoal cohesion, or decreasing foraging and overall activity (Chivers & Smith, 1998; Ferrari et al., 2005; Kelley & Magurran, 2003; Wisenden et al., 2004). Many fish species exhibit a strong learned recognition of predators via pairing of conspecific (or even sympatric heterospecific) alarm cue with visual, chemical, or mechanical predator cues (e.g., Brown, 2003; Holmes & McCormick, 2010; Lönnstedt & McCormick, 2011; Manassa, Dixson, McCormick, & Chivers, 2013; Mathis & Smith, 1993; Wisenden, Vollbrecht, & Brown, 2004). Fathead

minnows (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss)*, and brook trout (*Salvelinus fontinalis*), for instance, display recognition of a predator following only a single learning event, comprised of a pairing of alarm cue and predator odour (Brown, 2003; Brown & Smith, 1998; Chivers & Smith, 1994b; Mirza & Chivers, 2000). Moreover, fathead minnows can learn risk in a concentration-dependent manner, whereby higher concentrations of either alarm cue or predator odour (suggesting a high risk) invoke a greater intensity of response than lower concentrations (suggesting a low risk) (Ferrari, Capitania-Kwok, & Chivers, 2006; Ferrari & Chivers, 2006). Alarm cue learning has been shown to induce robust predator learning and even enhance individual survival in subsequent predator encounters (Gazdewich & Chivers, 2002; Mirza & Chivers, 2000, 2001). Predator information learned by lab-trained minnows has been shown to be retained for upwards of two months after conditioning (Chivers & Smith, 1994a), whereas wild populations of fathead minnows removed from their environment and maintained in the laboratory retained their antipredator response to a known predator for up to a year (Chivers & Smith, 1994b). Across taxa and environmental conditions, however, predator information is retained for varying degrees of time (Brown & Smith, 1998; Chivers & Smith, 1994a; Ferrari, Brown, Jackson, Malka, & Chivers, 2010; Mirza & Chivers, 2000).

1.3 Generalization of Predator Recognition

Lacking prior experience and evolutionary history with a predator, prey are typically unable to immediately recognize a novel predator as a threat or exhibit effective or appropriate antipredator behaviour (Sih et al., 2010). Interestingly, some prey have demonstrated apparent recognition of novel predators without prior experience (e.g., Davis, Epp, & Gabor, 2012; Ferrari et al., 2007; Sih et al., 2010). Fathead minnows, for instance, display antipredator behaviour in response to the odour of novel predators so long as a closely-related species is a known predators (Ferrari et al., 2007). So far, this phenomenon, known as 'generalization of predator recognition', has only been observed in a limited number of species (e.g. tammar wallabies, Griffin, Evans, & Blumstein, 2001; blacktail deer, Stankowich & Coss, 2007; freshwater snails, Langerhans & DeWitt, 2002; fathead minnows, Chivers & Smith, 1994a; Ferrari et al., 2007; glowlight tetras, Darwish, Mirza, Leduc, & Brown, 2005; lemon damselfish, Mitchell, McCormick, Chivers, & Ferrari, 2013; larval woodfrog tadpoles, Chivers, Mathiron, Sloychuk, & Ferrari, 2015; Ferrari, Brown, Messier, & Chivers, 2009; velvet geckos, Webb, Du, Pike, & Shine, 2009), and thus, more research is required to delve deeper into the complexities surrounding generalization—in

different environments and general context—to understand the variability, similarities, and differences observed among species.

As summarized by Ghirlanda & Enquist (2003), animals will exhibit similar behavioural responses to novel stimuli that are analogous to a known stimulus. Recent studies have expanded this framework to incorporate antipredator behaviour in response to similar and dissimilar threatening stimuli. Instead of learning specific predators per say, prey may learn specific types of stimuli (e.g., morphological, functional, or physiological) to form a predator template, and then utilize that information to extrapolate or generalize to novel organisms that express similar stimuli. Tammar wallabies (*Macropus eugenii)* trained to fear a model red fox (*Vulpis vulpis*) exhibited antipredator behaviour not only to the fox when presented, but also to a model cat; this generalization was not extended, however, to a model goat (Griffin et al., 2001). The authors propose that the extent of visual learning specificity may have been mediated by similar functional cues between the fox and the cat, or rather morphological similarities of carnivores such a forward-facing eyes (Blumstein, Daniel, Griffin, & Evans, 1999). In aquatic environments, recognition of closely-related predators has also been shown through olfactory recognition. Fathead minnows, juvenile rainbow trout, lemon damselfish (*Pomacentrus moluccensis*) and larval woodfrog tadpoles (*Rana sylvatica*) have all shown the ability to learn a predator via pairing of conspecific alarm cue and predator odours, and further generalize to novel but phylogenetically-similar predators (e.g., Brown et al., 2011; Ferrari et al., 2009; Ferrari et al., 2007; Mitchell, Chivers, McCormick, & Ferrari, 2015; Mitchell et al., 2013).

Similar stimuli often share common causal mechanisms or similar evolutionary pathways in the natural environment (Ghirlanda & Enquist, 2003). We see animals approaching novel situation in similar ways, and using knowledge familiar to them in these novel conditions. Theoretically, predators that share similar foraging habits would likely feed on the same prey and release similar diet cues. In these instances, learning specific cues to create a predator template may prove beneficial in ecological time, both in increased survival probabilities and reduction of costs associated with learning. The overall cost associated with initial encounters would likely be reduced, as the prey would not have to learn through direct experience with every new predator encountered (Ferrari, Messier, & Chivers, 2008). Similarly, as learning is likely associated with an increased cost in neural development and capacity, generalizing similar cues would reduce this cost, allowing for energy to be directed towards other fitness-related activities (Brown et al.,

2011; Dewitt, Sih, & Wilson, 1998; Mery & Kawecki, 2016). The ability to plastically adapt to new predators by generalized predator recognition may further support the idea that continual assessment of a prey's local predation threat reduces susceptibility to predators (Brown et al., 2011; Davis et al., 2012).

Conversely, as beneficial as it would be to generalize to potential predators, it would likely be equally beneficial to generalize to non-predators, enhancing the decision-making ability for prey to solely respond to predation threats that are ecologically relevant (Lima & Dill, 1990). Responding to non-threatening experiences wastes necessary energy that could be put towards other fitness-related activities. Recognition of non-threatening species and generalizing to taxonomically-similar novel species would therefore reduce the cost associated with responding to non-threats (Brown et al., 2011; Mitchell, McCormick, Ferrari, & Chivers, 2011). In aquatic environments, various species have shown the ability to learn a non-predator via latent inhibition, or repeated exposure to a predator with no negative reinforcement (e.g., crayfish (*Orconectes virilis*; *Orconectes rusticus*), Acquistapace, Hazlett, & Gherardi, 2003; fathead minnows, Ferrari & Chivers, 2006b; lemon damselfish, Mitchell et al., 2011; larval damselfish, Chivers, McCormick, Mitchell, Ramasamy, & Ferrari, 2014; woodfrog tadpoles, Ferrari & Chivers, 2009). Generalization of this inhibition has received minimal attention, however. One recent study on olfactory predator recognition demonstrated that juvenile rainbow trout could learn that the odour of pumpkinseed sunfish (*Lepomis gibbosus*) was harmless through latent inhibition (Brown et al., 2011). During subsequent associative alarm-cue training sessions, those same rainbow trout failed to learn pumpkinseed as a predator, and further generalized this inhibition to the closely-related longear sunfish (*Lepomis megalotis*). This study by Brown et al. (2011) demonstrated that it is indeed possible for prey to generalize safety information about novel animals through chemosensory recognition. Similar results were found in trials using a visual stimulus in clear water (Chivers, Al-Batati, Brown, & Ferrari, 2013); fathead minnows pre-exposed to the sight of a predator and then conditioned to fear that predator subsequently showed no response to the sight of the predator nor to closely-related predators. Other species, however, have not demonstrated the same ability through visual stimulus—even with repeated exposure to novel threats, rhesus monkeys (*Macaca mulatta*) appear unable to learn safety via latent inhibition of visual stimuli alone (Mineka & Cook, 1986). This may be attributed to

previously developed predator templates, which may include functional and morphological traits of known predators.

As noted, there appears to be a clear fitness benefit for generalization to both novel predators and novel non-predators. Responding to ecologically relevant predation threats would allow more energy and time to be invested in other fitness-related activities. Generalization, however, is not always the most reliable mechanism for identifying novel predators. Mitchell et al. (2015) found that in diverse coral reef ecosystems, lemon damselfish were able to effectively learn to recognize predatory moon wrasse (*Thalassoma lunare*) odour as a threat, and further generalized this recognition to other congeneric species. Based on chemosensory information alone, however, damselfish could not distinguish between the non-predatory blunt-headed wrasse (*Thalassoma amblycephalum*) and the predatory sixbar wrasse (*Thalassoma hardwicke*). Similar findings were observed in studies investigating velvet gecko (*Oedura lesueurii*) antipredator responses—velvet geckos generalized their predator recognition to the odours of both non-predatory and predatory venomous snakes, regardless of the actual threat level that each elapid species posed (Webb et al., 2009; Webb, Du, Pike, & Shine, 2010). Perhaps these species come from communities with high predator to non-predator ratios (i.e., more predatory species than non-predatory species within a taxonomic group). In these instances, it would then be safer to generalize to all similar species than take the chance of not responding to a predator. Generalized predator recognition would then encompass a wider range of species, given the ecological and taxonomic similarities of the predators (Chivers et al., 2014; Ferrari et al., 2007; Ferrari, Messier, & Chivers, 2008; Mitchell et al., 2013; Webb et al., 2010).

1.4 Recreational and Conservational Fish Stocking

Purposeful stocking of fish have been recorded in Canada and the U.S. as early as the 1800's. With little to no consideration for ecological consequences, its sole purpose was to enhance recreational sport fishing (Fausch, 1988; Knapp, Corn, & Schindler, 2001; Pister, 2001). Today, although more attention is given to the receiving ecosystems, stocking remains largely fueled by recreational desires while minimal stocking, in comparison, is performed to combat conservation of threatened species (Eby, Roach, Crowder, & Stanford, 2006). Exotic species as well as anthropogenically-hybridized fish are being introduced to increase the allure of annual stocking programs, which may result in an above-normal quantity and/or composition of predator populations. This non-native predator enhancement can have a significant effect on prey

abundance, survivorship, foraging behaviour and habitat choice as well as predator genetic variation (Allendorf, Leary, Spruell, & Wenburg, 2001; Eby et al., 2006; Kats & Ferrer, 2003). In Saskatchewan, for instance, it has been suggested that stocking of non-native salmonid species have contributed to the complete absence of the threatened mountain sucker (*Catostomus platyrhynchus*) in certain areas of their native range in Battle Creek (Boguski & Watkinson, 2013). In extreme cases, introduced species can completely decimate native vertebrate and invertebrate prey populations (Knapp et al., 2001; Rahel, 2000). This reduced survivorship or significant population decline may result from a failure to recognize or appropriately respond to novel predators (Sih et al., 2010). However, behavioural plasticity and generalization of predator recognition may pose a significant fitness advantage in the event of novel fish introductions or invasions, as strict reliance on innate recognition may increase prey susceptibility (Gall & Mathis, 2010). Furthermore, animals that base their response on experience or generalize from known predators might be better equipped to recognize novel predators, as they continually update predation risk and information to present conditions. This may consequently increase their likelihood of responding appropriately to novel stimuli.

Prey naïvety appears to be even less of a concern with the introduction of hybrid predators (Allendorf, Leary, Spruell, & Wenburg, 2001; Chivers et al., 2015). Hybrids (potentially exhibiting chemical signatures of both parental species) may provide prey with an even greater survival advantage if one or both of the parental species are known predators. Only one known study has observed predator generalization to novel hybrid predators. Chivers et al. (2015) trained larval woodfrogs to identify brown trout (*Salmo trutta*) as a predator. When tested for recognition of brown trout and generalization to tiger trout and brook trout, the tadpoles exhibited the strongest response to the learned predator, with an intermediate response to hybrid, and the least intense response to the closely-related brook trout. Prey may, therefore, be better equipped to respond effectively given the cue similarity of the novel hybrid and known parental species.

1.5 Research Objectives

The overall objective of my research was to delve deeper into understanding the extent of generalization of predator recognition, specifically in the context of hybrid predator stocking and introduction. My research was investigating one facet of the hypothesis that generalization of predator recognition is influenced by the phylogenetic distance between known and novel

predators. This hypothesis predicts that prey will decrease their intensity of antipredator response as the phylogenetic distance increases between the known and novel predators. In my thesis, I present two data chapters examining how a model prey species, with knowledge of different trout predators, responds to novel hybrid trout predator odours. These results are aimed to help us understand what characteristics may need to be considered prior to stocking. Specifically, I used an aquatic model species (fathead minnows) to answer the following questions:

Does knowledge of a parental species impact the ability for prey to identify novel hybrid predators? If so, how does the generalized response to the hybrid odour compare to that of the known parental odour and the unknown parental odour? My first experiment (Chapter 2) explores the potential cue similarities of hybrid predators to their parental species (paternal—brook trout; maternal—brown trout). This was accomplished by conditioning minnows to recognize one of the parental species via alarm cue learning. The intensity of response exhibited by minnows was then compared when tested for recognition of the known parental odour, the novel confamiliar parental odour, the hybrid tiger trout odour, and a distantly-related predatory species (northern pike, *Esox lucius*). Prior to this research, only woodfrog tadpoles had been tested for their ability to generalize recognition to a hybrid tiger trout after knowledge of parental species (Chivers et al., 2015). In that experiment, however, the design of the study did not allow for the researchers to compare the intensity of response to the known and novel odours. My first experiment was designed as a follow-up to this study to observe: 1) if the patterns of generalized predator recognition to hybrid odours were consistent across these aquatic taxa, and 2) how the response to the novel odours compared with the response elicited by the known parental odour.

Does knowledge of both parental species influence the ability of prey to generalize recognition to the hybrid predator? In Chapter 2, I wanted to understand whether knowledge of both parental species might pose a benefit to prey species. Minnows were conditioned to learn both parental odours (brook and brown trout) as predators, and then tested with the odour of each parental species, the hybrid tiger trout, and the distantly-related northern pike. Minnows were observed to determine whether knowledge of both parental odours would intensify the behaviour elicited from exposure to the novel hybrid odour.

Would knowledge of a non-parental trout species facilitate generalized recognition to hybrid trout odours? In Chapter 3, I investigate whether knowledge of a confamiliar trout species (rainbow trout) to the parental species would allow prey to generalize recognition to the

novel hybrid odour. Thus, I further investigated whether phylogenetic relatedness influenced generalization of predator recognition by incorporating a non-parental trout species into my experimental design. To answer these questions, I conditioned minnows to learn either a parental species of the tiger trout (brook trout) or a non-parental species (rainbow trout). Antipredator behaviour was then observed when exposed to the odour of the known predator, the hybrid tiger trout, the confamiliar trout species, and a distantly-related northern pike.

What response do minnows initially exhibit towards known and novel odours, and how do those responses change over time? Antipredator responses, such as increased shoaling, freezing, and decreased activity (e.g., feeding, movement, etc.) are commonly used as behavioural assays to study changes in behaviour associated with potential predation threats. Throughout my thesis, I chose to analyze this information in two different ways: 1) as an overall change in response, incorporating behavioural data from across the entire observation period, and 2) broken down into time segments, examining the change in response over two time segments (early response period—initial response to the odour; late response period—response to odour after 6-min). Analysis of the overall change in response would allow my results to be comparable with other studies investigating the generalization of predator recognition in fishes, as this interpretation of data is most commonly employed in this field of study. Overall, I predict that a graded antipredator response will be observed, with the highest intensity response being elicited by the known predator odour. Response intensity to novel odours will then decrease as the phylogenetic distance between the known and novel predator increases. Analyzing the response over time, however, has received less attention. According to the threat-sensitive predator avoidance hypothesis, the intensity of antipredator response exhibited by prey typically correlates with the level of threat posed by the predator (e.g., Ferrari, Messier, & Chivers, 2008; Ferrari, Capitania-Kwok, & Chivers, 2006; Ferrari & Chivers, 2006a). Following this line of thought, I predict that: 1) the highest intensity of response would be exhibited towards the highest threat (i.e., the known predator and closely-related predators) at each time segment, and 2) the highest intensity of response to each odour would be immediately following the odour introduction, and then would decrease over time—the impeding threat would likely decrease over time without additional reinforcement (Zhao, Ferrari, & Chivers, 2006). Although there may be an initial response to distantly-related species, I predict that only odours representing high-threats will elicit a sustained response into the late observation period.

1.6 Anticipated Significance

The findings of my research may provide crucial information to consider during ecological assessments and recommendations, as well as for analyses of ecosystem susceptibility to introduced or invasive species, especially prior to fish stocking. Introduced and invasive species often have a strong, negative impact on prey survival (Davis et al., 2012). These effects may be minimized with careful consideration of the species currently present in the ecosystem and those being introduced. My research may indicate the relative ease with which prey can generalize from a known predator to hybrid and closely-related introduced predators. If looking for exotic fishes for recreation, stocking hybrids (potentially exhibiting chemical signatures of both parental species) may further provide prey with a substantial survival advantage if one or both of the parental species are known predators. For instance, stocking sterile tiger trout, a human-mediated hybrid of a female brown trout and a male brook trout, may maintain ecological stability without altering the predator gene pools (Allendorf et al., 2001); prey may be better equipped to respond effectively given the cue similarity of the novel hybrid and known parental species.

Although it is possible to regulate fish being stocked to specific ecosystems while accounting for local species diversity (i.e., evenness and richness), it is close to impossible to prevent invasive species from entering a landscape. If we can better understand the current mechanism by which an organism identifies predators, and further generalizes to novel predators, we may be better equipped to predict how non-native predators may affect an invaded community (Sih et al., 2010). Furthermore, knowledge of prey vulnerability, especially in the case of threatened organisms, will allow resource managers to focus on a specific habitat prior to potential invasion. Understanding how prey generalize various predator characteristics may help to illuminate how organisms will respond to novel introduced predators. It may then be possible to better predict and protect different ecosystems from the impacts of introduced and invasive species.

Chapter 2: Influence of parental species odour(s) on generalized predator recognition of a novel hybrid predator

2.1 Introduction

Declining fisheries and recruitment of native populations have generated significant concern for fisheries and conservation biologists. The variability in reproductive success from year-to-year further complicates the instability of certain fish populations (Minto, Myers, & Blanchard, 2008). In order to assist in the conservation of ecosystem health, human-mediated introductions have been instrumental for the rejuvenation or enhancement of declining species, establishment of new fisheries using species of interest, and providing enhanced opportunities for recreational anglers (Saskatchewan Ministry of Environment, 2013). Tiger trout introductions in Saskatchewan are one example of the establishment of new fisheries to satisfy the interest of anglers and/or stakeholders. In 1988, tiger trout were experimentally introduced into six lakes, but were not stocked in significant numbers until the early 1990's (C. Prestie, Fisheries Management Biologist, Government of Saskatchewan, Personal Communication), likely due to increasing angler interest.

Although the majority of hybrid tiger trout around the world have been stocked with fish reared in hatcheries, there have also been accounts of natural hybridization between brook trout and brown trout. Where habitat overlap occurs, few studies have observed interactive behaviour between these confamiliar species, showing evidence for overlap in spawning season as well as physical attempts to breed (Cucherousset, Aymes, Poulet, Santoul, & Céréghino, 2008; Grant, Vondracek, & Sorensen, 2002; Sorensen, Cardwell, Essington, & Weigel, 1995). These interactions can sometimes come as a detriment to the sympatric species, as reproductive interference reduces each population's reproductive capabilities. In other instances, this interspecific reproduction and hybridization may impact the resident prey species, depending on their ability to recognize the novel hybrid predator. In the light of climate change, the warming of aquatic ecosystems is predicted to expand geographical ranges of temperate species, while those of coldwater species will shrink (Lynch et al., 2016). As ranges expand, it is likely that the

probability of hybridization of fish species will increase (Lynch et al., 2016). This increase in abundance of hybrid predators, either human-reared or naturally occurring, poses enhanced concern for conservation, sustainable fisheries, and maintenance of ecosystem health. Further understanding of how stocking and climate change will continue to impact the freshwater systems will be crucial to maintain environmental, cultural, and economic benefits of the fisheries industry.

Acquired predator recognition has been shown to facilitate behavioural adaptations to current environmental threats (Chivers & Ferrari, 2013; David, Salignon, & Perrot-Minnot, 2014; Ferrari & Chivers, 2006a; Ferrari et al., 2005). The ability to generalize predator recognition from a known predator to a closely-related novel species may also enhance survival possibilities during initial encounters with novel predators (Davis et al., 2012). Furthering our understanding of how prey may respond to these novel hybrid predators may help influence fisheries management decisions and predict how increased hybridization may impact the aquatic ecosystem. The ability for prey to respond may depend on the extent to which the hybrid predators' odour reflects its parental species' odours. If the odours are similar, knowledge of one or both parental species may enhance the prey's ability to recognize it as a predator on the first interaction. If not, however, the prey may have a difficult time recognizing this novel hybrid predator and thus, suffer significant fitness costs (Sih et al., 2010). Information pertaining to which outcome may be more likely will greatly impact conservation and stocking efforts.

To better understand this aspect of hybrid predator recognition, my research was designed to explore the potential cue similarities of hybrid predators to their parental species. This was done by investigating the extent to which fathead minnows would generalize their recognition to a hybrid predator after alarm-cue learning of odours from one or both of the parental species. For this experiment, my hybrid of choice was the tiger trout—the offspring created from the fertilization of female brown trout eggs by male brook trout milt. Minnows were conditioned to learn either brook trout (paternal species) or brown trout (maternal species) as a predator, and then tested for recognition of brook trout, brown trout, tiger trout, and a distantly-related species, the northern pike. In doing so, my research investigated the hypothesis that phylogenetic distance between a known predator and a novel predator influences generalization of predator recognition. It was predicted that this would manifest as a graded antipredator response, with the highest intensity response to the known predator and gradually

lower responses as phylogenetic distance between the novel species and the known predator increases. In this experiment, that would mean the highest intensity response would be to the known parental odour, with less response to the mixed odour signatures of the tiger trout, even less to the unknown parental odour and finally no generalization to the more distantly-related pike. Furthermore, I predicted a similar graded response to be observed in the time-series analysis—the odours that pose the highest threat (i.e. the known parental predator odour and the hybrid tiger trout odour) would elicit the highest intensity response during the early response period and that this response would be maintained or slightly decreased into the late response period. Odours of more distantly-related species may elicit a response during the early response period; however, I predicted that this response would drop off more substantially during the late response period, eliciting no significant increase in antipredator behaviour. Two underlying assumptions of this experiment are that the odour signatures of the fish reflect phylogenetic relatedness, and parental species' contribution to hybrid odour is relatively equal.

2.2 Methodology

2.2.1 Test Species – Collection and Maintenance

Fathead minnows were collected from Feedlot Pond (surface area: ~10,200 m^2) on the University of Saskatchewan, North Management Area. This ecosystem is habitat for avian and invertebrate predators of minnows; however, with the absence of predatory fish, these minnows are naïve to fish predators. Various studies have shown that this minnow population has demonstrated a learned recognition and response to predatory fish through a single alarm-cue learning event (Crane & Ferrari, 2015; Ferrari, Capitania-Kwok, et al., 2006; Ferrari et al., 2005). For retrieval, Gee's inverted minnow traps (cylindrical traps with conical entrances) were left overnight and checked daily. Minnows then acclimated for at least three weeks in our standard 37 L tanks (50.3 x 25.3 x 30 cm) (~15°C) containing an air stone, gravel substrate, and a water filter. Minnows were fed flake food *ad libitum* and maintained under a 14:10 h light:dark (l:d) cycle.

2.2.2 Stimulus Preparation

Fathead Minnow Alarm Cue

Standard procedure was used for making alarm cue (Darwish et al., 2005; Ferrari & Chivers, 2006b). Five minnows were randomly selected and killed with a blow to the head (in

accordance with the Canadian Council on Animal Care). Skin filets were removed from the lateral sides of each minnow, placed in 20 mL of dechlorinated tap water, and homogenized (Polytron PT-2500E). Filtered alarm cue solution was diluted, according to an established protocol, to obtain a concentration of ~1cm^2 of skin/40 L of water (Ferrari, Capitania-Kwok, & Chivers, 2006; Ferrari & Chivers, 2006a, 2006b; Ferrari et al., 2005). The alarm cue solution was divided and frozen (-20°C) in 100 mL portions.

Predator Odour

The brook, brown, and tiger trout were maintained at the Cold Lake Fish Hatchery (AB, Canada), separated by species, and fed a diet of trout pellets. Northern pike were caught from Pike Lake (SK, Canada) using a seine net. Pike are strictly piscivorous so they were fed minnows prior to stimulus collection. Following established protocol, arbitrarily chosen, size-matched pike (mean ± S.E. total length (TL) = 209.4 ± 4.1 mm) were moved to separate 37 L tanks, eight days prior to stimulus collection (Ferrari et al., 2007). Potential conspecific diet cues were removed by feeding pike two swordtail (*Xiphophorus hellerii*) per day leading up to stimulus collection (Ferrari, Messier, & Chivers, 2006; Mathis & Smith, 1993). Forty-eight h prior to stimulus collection, size-matched brook trout (mean ± S.E. TL = 210.9 ± 1.5 mm), brown trout (mean ± S.E. TL = 204.5 ± 1.5 mm), and tiger trout (mean ± S.E. TL = 207.7 ± 0.8 mm) were chosen and moved to identical tanks with clean dechlorinated water. All predators were starved for 48 h to allow for maximum gastric evacuation before collection began (Bromley, 1994; He & Wurtsbaugh, 1993; Nilsson & Brönmark, 2000). Fish were then transferred into collection tanks filled with a volume of clean dechlorinated water relative to the size of each fish (50 mL of water/g of fish). Predators were left for 24 h with no water replacement, removed and then fed once returned to original holding tanks. Predator-conditioned water was mixed, divided into 100 mL portions, and frozen (-20°C) until needed.

2.2.3 Experimental Protocol

My experiment followed a 3x4 design. Minnows were conditioned to learn the odour of (A) brook trout (BkPO), the tiger trout paternal species, (B) brown trout (BnPO), the tiger trout maternal species, or (C) both brook and brown trout. The four test odours used for recognition were (1) BkPO, (2) BnPO, (3) the hybrid tiger trout (TgPO), or (4) distantly-related northern pike (PiPO) (negative control).

Conditioning Exposure

After three weeks of laboratory acclimation, minnows were moved into experimental tanks in random groups of 3 fish per tank, equipped with dechlorinated tap water (~15°C), gravel substrate, an air stone, and an injection tube, situated closely to the air stone (Fig. 2.1). Minnows were left to acclimate for 48 h under the same environmental conditions as before (i.e., temperature, l:d cycle). Conditionings entailed pairing of 20 mL of predator odour (PO) with 5 mL of conspecific alarm cue (AC). Each tank was randomly assigned one of three conditioning treatments: (A) BkPO followed by AC, on Day 1 and 2, (B) BnPO followed by AC, on Day 1 and 2, or (C) BkPO followed by AC on one day; BnPO followed by AC on another day. For conditioning treatment (C), the order of BkPO and BnPO was randomized over the concurrent days to observe for a potential order effect on learning and/or recognition.

Minnows were fed 1 h before conditioning. Immediately prior to conditioning events, 60 mL of water was extracted from the injection tube, to remove any stagnant water, and discarded. Another 60 mL was then extracted and retained. After the respective PO & AC injections were administered via the injection tube, the 60 mL of tank water was re-injected to flush any remaining cue into the conditioning tank. An hour after the conditioning event, conditioning tanks underwent a gradual 75% water change, and fish were fed. After 24 h, minnows were conditioned with the second conditioning event, following the same procedure as described above. One hour after the final conditioning, the minnows were moved to identical 37 L testing tanks with clean dechlorinated water and fed.

Recognition Trials

Fish were fed 23 h after final conditioning; recognition trials began 1 h after feeding. Before the stimulus injection, 60 mL of tank water was removed through the injection tube, to clear stagnant water, and discarded. Another 60 mL was removed and retained. One minnow in each tank was randomly selected and observed continuously for 8-min directly prior to the stimulus injection. The number of lines crossed (using a 3 x 3 grid pattern mapped on the tank) by the observed minnow was recorded for every 15 s throughout the observation period. Following the pre-stimulus observation, 20 mL of BnPO, BkPO, TgPO, or PiPO was injected then flushed with the retained 60 mL of tank water to introduce the entirety of the stimuli. Post-

stimulus 8-min observation commenced immediately after introduction, following the same procedure as above.

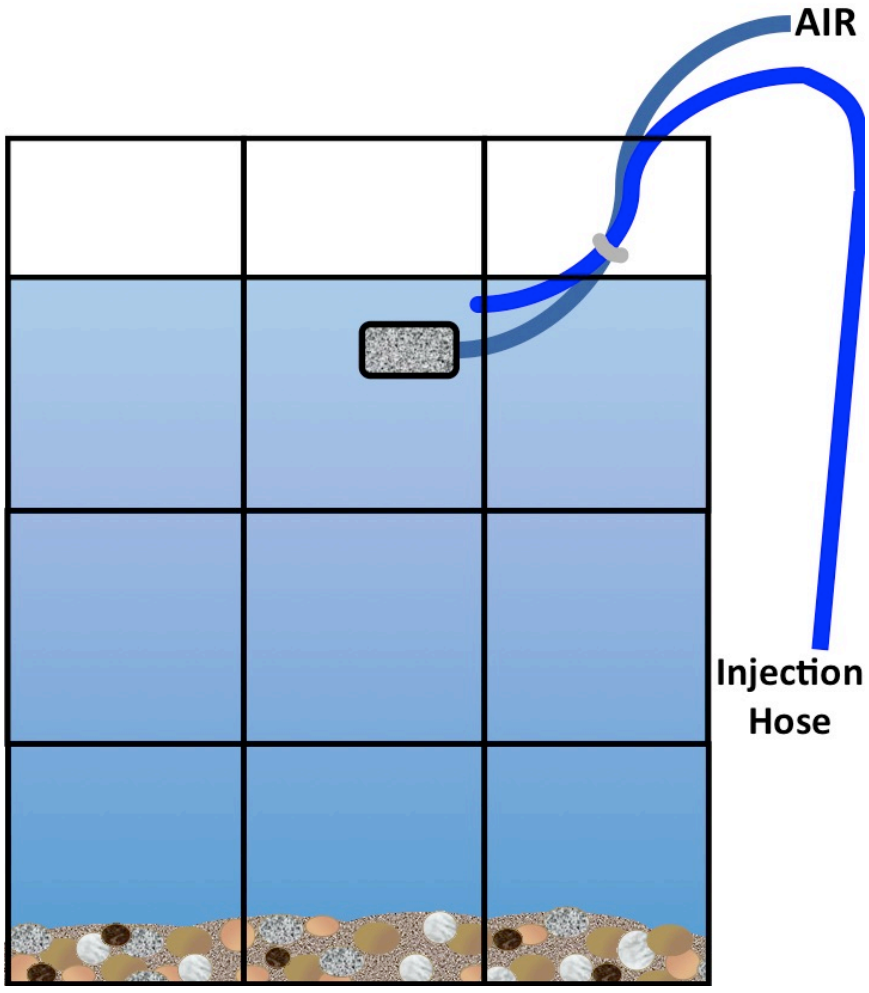

Figure 2.1: Schematic diagram (side view) of test tanks used in Experiments 1 and 2.

2.2.4 Statistical Analysis

Change in Overall Activity

Pre-stimulus baseline activity levels for treatment group combinations were analyzed using a 3x4 ANOVA, with (1) BkPO, (2) BnPO, or (3) BkPO & BnPO as the three conditioning groups and (A) BkPO, (B) BnPO, (C) TgPO, and (D) PiPO as the test groups. The total number of lines crossed for pre- and post-stimulus activity were computed to a change in line crosses (total # post-stimulus line crosses minus total # of pre-stimulus line crosses) and used as the response variable for analyses. Data from change in line crosses were rank-transformed to

account for the unequal variances and analyzed using a non-parametric 2-way ANOVA (Scheirer-Ray-Hare extension of the Kruskal-Wallis test, Sokal and Rohlf 2003). Significant differences were analyzed using Tukey HSD post hoc tests.

Early versus Late Response

We further investigated the mean line cross differences over a time series, to observe any changes in response over the 8-min post-stimulus observation period. Two time segments were selected for analysis—an early response period, defined as the first 2-min after the stimulus was injected, and a late response period, defined as the last 2-min within the 8-min post-stimulus observation period. As with prior analyses, change in line crosses was calculated for each time segment by subtracting the mean number of lines crossed during the pre-stimulus observation period from the mean number of lines crossed during the post-stimulus observation period. Data were log-transformed to account for heteroscedasticity. Mean change in line crosses was analyzed using a 2x3x4 repeated measure ANOVA on the log-transformed data, with time (early, late) as the within-subjects variable, and the conditioning and testing odours as the between-subjects variables. Data were separated into conditioning groups, and differences in mean change in lines crossed between the four test odours were compared within each time segment (early, late) using a 1-way ANOVA. For treatments where both brook and brown trout odour were used to condition, the order of conditioning was incorporated as a factor in the analysis to observe any potential effect on behaviour. Significant differences were analysed with Tukey HSD pairwise post hoc comparisons. The statistical analysis was carried out using IBM SPSS Statistics 22 with statistical significance set as $\alpha = 0.05$.

2.3 Results

Change in Overall Activity

Minnows displayed the same pre-stimulus baseline activity levels regardless of treatment group (test: $F_{3, 260} = 1.8$, $p = 0.2$; conditioning: $F_{2, 260} = 1.1$, $p = 0.3$; interaction: $F_{6, 260} = 0.8$, $p = 0.6$), crossing, on average, 15 lines per minute. Change in line crosses was not affected by an interaction between conditioning odour (BkPO, BnPO, both) and testing odour (BkPO, BnPO, TgPO, PiPO) (non-parametric ANOVA: $H_{6, 260} = 0.7$, $p = 0.7$), nor did it differ between conditioning odours ($H_{2, 260} = 0.2$, $p = 0.8$). However, the behaviour of minnows was affected by the odour they received during testing ($H_{3, 260} = 29.5$, $p < 0.001$). Minnows responded similarly to

the three trout odours (Tukey HSD: all p >0.23), with all trout odours eliciting a stronger antipredator response than the pike odour control (all p<0.001) (Fig. 2.2).

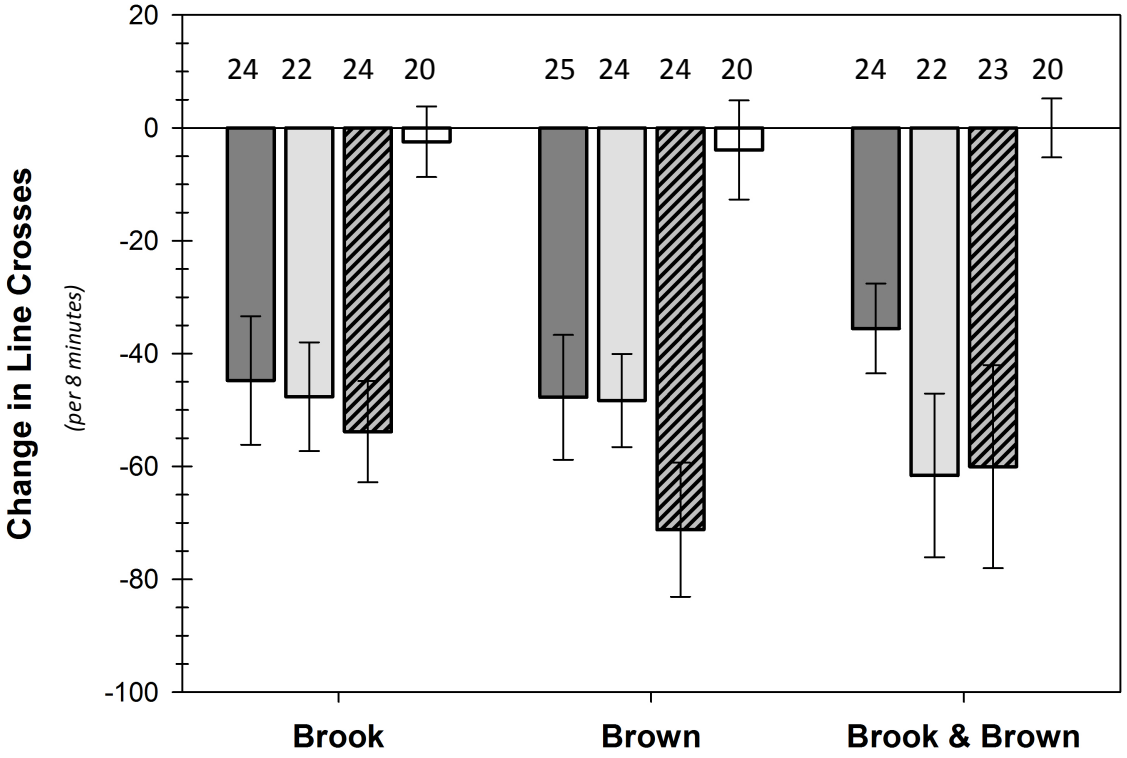

Figure 2.2: Mean (±S.E.) change in line crosses from the pre-stimulus baseline for minnows conditioned to learn brook trout, brown trout, or brook and brown trout as a predator. Minnows from each conditioning group were then tested for recognition of brook trout (dark gray bars), brown trout (light gray bars), tiger trout (gray stripped bars), or northern pike (white bars) odour. The value above each bar indicates sample size.

Early versus Late Response

When observing the time series, there was no significant 3-way interaction among time (early, late), conditioning odour (BkPO, BnPO), and test odour (BkPO, BnPO, TgPO, PiPO) on lines crossed (3-way RM ANOVA: $F_{6, 260} = 1.2$, $p = 0.3$). However, lines crossed were affected by an interaction between time and conditioning odour ($F_{2, 260} = 3.4$, $p = 0.04$), as well as by the predator odour presented during testing ($F_{3, 260} = 9.2$, $p < 0.001$).

When minnows were trained to recognize brook trout as a predator, test predator odours elicited different responses during both the early response period ($F_{3, 86} = 3.4$, $p = 0.02$) and the late response period ($F_{3, 86} = 3.7$, $p = 0.01$). Minnows exposed to the known predator, brook trout, responded with greater intensity than those exposed to the pike odour control (Early—$p = 0.03$;

Late—p = 0.009), while the other two trout odours elicited intermediate responses to those exposed to brook and pike odours (see Fig. 2.3a for post-hoc comparisons). These responses were maintained throughout both response periods.

When conditioned to recognize brown trout as a predator, minnow responses differed for the four test predator odours during the early response period ($F_{3, 89} = 3.7$, $p = 0.02$) and the late response period ($F_{3, 89} = 3.6$, $p = 0.02$). Initially, minnows tested with brook, brown, and tiger trout responded similarly (all $p > 0.9$) and with greater intensity than those tested with the pike odour control (all $p < 0.05$) (Fig. 2.3b). This pattern changes, however, during the late response, with sustained high-intensity response to the brown and tiger trout odours but with a reduction in response to the brook trout odour. Responses elicited by brown and tiger trout remained significantly greater than responses elicited by the pike odour control (all $p < 0.04$), while the intensity of response elicited by the brook trout decreased to an intermediate response.

When conditioned with information about two predators (brook trout, brown trout), there was immense variation in the response variable data for the early and late response periods (Levene's: Early—$F_{3, 85} = 4.8$, $p = 0.004$; Late—$F_{3, 85} = 10.3$, $p<0.001$). After analyzing the order of conditioning, it was found that order had no significant effect on minnow behaviour during either response periods (Early—$F_{1,85} = 22.3$, $p = 0.7$; Late—$F_{1, 85} = 11.4$, $p = 0.8$); therefore, the variation in raw data was not caused by a dichotomous response between groups that differed in their conditioning orders. Further analysis of log-transformed data indicated that while initially there were no differences in response elicited by the four test odours during the early response period ($F_{3, 85} = 2.1$, $p = 0.1$), responses changed significantly during the late response period ($F_{3, 85} = 2.1$, $p = 0.001$) (Fig. 2.3c). Strong sustained responses were only evident in minnows tested with brook and brown trout odour, the two known predators. Minnows exposed to pike odour control significantly diminished their response in comparison to the known trout predators (all $p <0.006$) whereas those exposed to tiger trout odour reduced their response to an intermediate level.

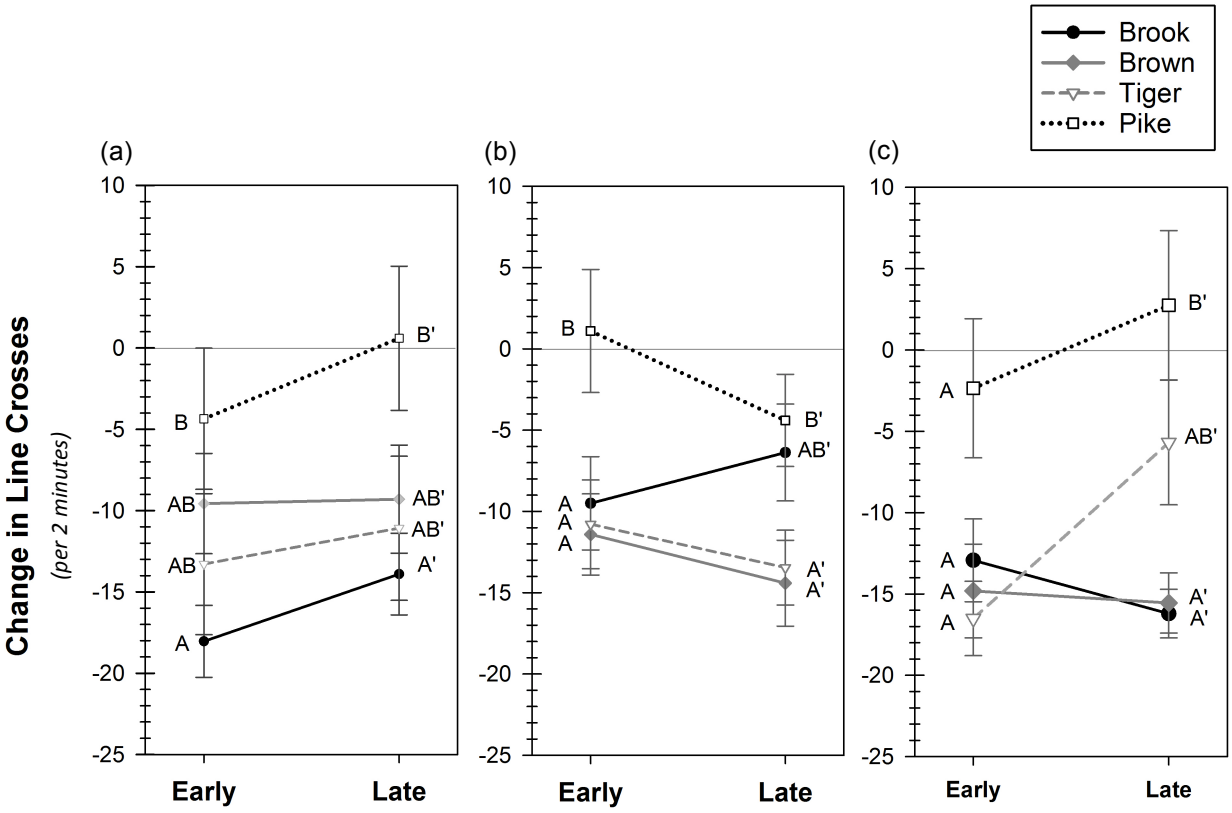

Figure 2.3: Mean (±S.E.) change in line crosses from pre-stimulus baseline during early and late response periods for minnows conditioned to recognize (a) only brook trout, (b) only brown trout, or (c) both brook and brown trout as predators. Minnows from each conditioning group were then tested for recognition of brook trout (black lines), brown trout (light gray lines), tiger trout (gray dashes lines), or northern pike (black dotted lines) odour. Letters indicate statistical significance (α = 0.05). Sample sizes were 20 to 25 per group.

2.4 Discussion

My results demonstrate that fathead minnows have the ability to generalize predator recognition from a known predator to confamiliar predators. Furthermore, generalization of predator recognition was extended to a novel hybrid predator when one or both of the parental species were known predators. Interestingly, my results also highlighted the effect of time on generalized responses; the duration of response to the confamiliar species and the hybrid species odours varied with conditioning odour over time. The intense response to the known predator, however, was sustained across the observation period.

Whether the minnows were trained to learn brook trout, brown trout, or both trout as predators, the overall responses during the experiments were no different—knowledge of one or both parental species as predators led to a significant antipredator response to all three trout odours during testing. Generalization was extended to the odour of confamiliar predators, supporting previous findings in San Marcos salamanders (*Eurycea nana*), fathead minnows, and

woodfrog tadpoles (Davis, Epp, & Gabor, 2012; Ferrari, Gonzalo, Messier, & Chivers, 2007; Ferrari, Messier, & Chivers, 2008; Ferrari, Crane, & Chivers, 2016). However, in my experiment, the similarity of response when exposed to the known predator, the hybrid predator, and confamiliar species was unexpected. Intensity of response during generalization of predator recognition has been shown in a variety of fish species to typically correlate with the degree of taxonomic similarity between the novel odour and the known odour, with a decrease in intensity as taxonomic similarity decreases (e.g., Brown et al., 2011; Ferrari et al., 2007, 2008; Mitchell et al., 2013). Only a few studies have shown a hypersensitive response, whereby prey show no apparent discrimination between closely-related species within the same genus (Brown et al., 2011; Mitchell et al., 2013). Further investigation will be necessary to understand what may influence similar response intensities elicited by confamiliar species.

Fathead minnows were able to extend generalization of predator recognition to the hybrid tiger trout from knowledge of one or both of the parental species. This response is consistent with what has been observed in larval woodfrogs (Chivers et al., 2015). These results suggest that tiger trout may indeed, share a similar suite of odours, or concentrations of specific odour(s), with the parental species. Interestingly, when Chivers et al. (2015) conditioned tadpoles to fear tiger trout and then tested for recognition of both parental species, the intensity of response was greater for the brown trout than for the brook trout odour. In subsequent trials, antipredator response to tiger trout was strongest when tadpoles were trained with at least one conditioning of the maternal species, brown trout. Although my results showed minnows did not differentiate their response to the three trout odours, results displayed in Fig. 2.2 suggest that there is an observable trend for a more intense response to the tiger trout when the maternal species is a known predator. The results of these two studies suggest that, genetically, both parents may not contribute equally in the production of offspring odour.

Mechanistically, there is still much to be explored in regards to generalization of predator recognition. It is possible that minnows are detecting specific odours common to the known and the novel species or perhaps different concentrations of specific odours, and those concentration differences provide the prey with a relative indication of danger. Given that generalization was not extended to predatory pike, my results support our current knowledge that phylogenetic closeness of the trout, as opposed to general odours of predatory fishes, influenced the odour recognition and generalization in minnows. Since all trout odours elicited a similar antipredator

response, regardless of which predator(s) were known, it may be possible that they are responding to specific odours common to all trout. In the case of brown trout and brook trout, these confamiliar species have similar foraging strategies and feed on similar prey, thus it may be likely that they have similar biochemistry, undergo similar physiological processes and release similar odours (Chivers & Ferrari, 2013; Ferrari, Brown, Bortolotti, & Chivers, 2010; Ferrari & Chivers, 2009; Losos, 2008). To see if this reasoning extends to all trout with these ecological similarities, we would need further investigation to confer whether this response is maintained when non-parental trout odours are the known predator for instance (see Chapter 3).

Creating a predator template based on specific cues may be more complicated than previously thought and raises a few questions: Do these similarities in odour arise from a common evolutionary pathway, and thus are reflected in phylogenetic closeness? Do they arise given the ecological similarity of the predators? Or is it perhaps a combination of both? And if so, does one factor have more influence than the other? Mitchell et al. (2015) have recently demonstrated that generalization of predator recognition based solely on phylogenetic closeness may in fact result in antipredator responses when exposed to non-threatening congeneric species—a response that would constitute a waste of energy from a fitness perspective. This study was able to illuminate the important information that can be gained from predator odour as well as other ecologically relevant factors, such as diet cues. The interactions of these factors may strongly influence a prey's ability to accurately generalize predator recognition. In theory, predators with similar ecology, including feeding strategy and prey preference, would likely produce similar diet cues. Response to these cues may prove beneficial by reducing the cost associated with learning and increasing survival probabilities during initial encounters (Mirza & Chivers, 2003). However, as Mitchell et al. (2015) found, diet cues alone (without conspecific alarm cues present) were not responsible for the observed generalization. Going forward, it is important to remember that phylogenetic relatedness does not always constitute ecological similarity (see Sih et al., 2010; Sih, Ferrari, & Harris, 2011). If we perform our studies using only standardized diets, removing cues that would likely be present in natural populations, laboratory-based experiments may lose some relevance to natural ecosystems. It will be important for future studies to ensure that the presence of both phylogenetic relatedness and ecological niche have been accounted for, as each may influence prey's ability to generalize predator recognition in their natural environment. Future research should continue to examine

the influence of both phylogenetic relatedness and ecological similarity in reference to generalization of predator recognition.

The time-series analyses brought to light hidden patterns in the observed responses. As expected, the known predator odour(s) elicited the strongest change in behaviour; initial fear response was sustained throughout the observation period, indicating the high level of threat perceived by the minnows. When minnows recognized brook trout, odours of tiger trout and brown trout elicited an intermediate antipredator response to that of the known brook trout and the distantly-related pike. The generalized response is indicative of the recognition and assessment of tiger trout and brown trout as threatening; however, with an intermediate response to the known and the distantly-related species, it appears that these two odours were not as threatening as the known predator. Although not significantly different, response to the tiger trout was characteristically more intense than the response to the brown trout during both the initial and the late response periods, with a 41% reduction in activity when exposed to tiger trout and 31% reduction in activity in response to brown trout.

These intermediate responses may indicate that the novel odours were similar enough to the known predator odour to cross the behavioural response threshold, or the level of stimuli needed to elicit an overt behavioural response; however, the reductions in activity towards the novel salmonid odours were not as strong as the reduction of activity in response to the known predator. This threat-sensitive response pattern would indicate that the known predator was perceived as a higher threat, and thus a higher response was elicited (Brown et al., 2001, 2006). Perceived threat levels associated with novel odours, however, may have decreased due to increasing differences between the known and novel odour characteristics—observable by the pattern of a graded response. The observed responses to the novel salmonid odours may also indicate higher uncertainty about the threat associated with each odour. In instances of uncertainty, the perception of lower threat levels may influence minnows to exhibit different degrees of antipredator behaviours, some of which are not characterized by this study. It is possible that a slight decrease in activity, as shown here, would be accompanied with other antipredator responses, such as increased shoaling, decreased foraging, or increased refuge use, to approach these novel situations. The threshold for behavioural responses has been shown to vary among different antipredator responses (Brown et al., 2006), and thus, other behaviours may have been employed at lower thresholds when approaching novel odours. Further research

integrating multiple antipredator responses would help to identify how behavioural response thresholds differ between prey strategies when generalizing to novel stimuli.

Furthermore, tiger trout odour elicited a more similar response to the brown trout odour when minnows were trained to recognize this maternal species as a predator. Sustained fear response to the tiger trout odour was observed into the late response period, whereas the response to the paternal odour, the novel brook trout, was dropped to an intermediate response. These results provide a slight indication that there may in fact be a graded antipredator response, from the odour of the known parental to the hybrid and next to the confamiliar parental odours, which was hidden in the overall analysis.

It appears that knowledge of one of the parental species would provide a benefit to prey when novel hybrid predators are introduced into their habitat. With the ability to generalize to these theoretically similar smelling hybrids, the response is almost indistinguishable from the known predator. Unexpectedly, however, with knowledge of both parental species as predators, the sustained response to the tiger trout odour was not maintained. Initially, the strongest response was to the odour of the tiger trout—although not significantly different from any of the responses, activity in response to tiger trout odour was reduced by 59% in compared to 8% in response to pike odour. During the late response period, however, antipredator behaviour to the tiger trout odour became only three times stronger than that of the pike odour, potentially indicating the decrease in perceived threat associated with tiger trout odour over time. The responses to the parental species odours were maintained, with an almost six times stronger response than the response to the pike odour.

These results pose an important question—why would minnows decrease their response to the hybrid predator when both parental species are known predators? Theoretically, the combined odour signatures of the parental species are reflected in the tiger trout, and thus, if these conditioning events provided a reliable threat indication, knowledge of both should enhance the prey's ability to pick up signatures of each in the hybrid species odour. This change in perceived threat or propensity to reduce an antipredator response to the hybrid could be a result of the increased uncertainty surrounding the known predators. When low in number, the number of learning events have not been shown to affect the overall intensity of response to known and novel predators odours in the day following the last conditioning event; however, the effects of double conditioning have been shown to increase the number of days an antipredator

response is maintained for (Chivers & Ferrari, 2013; Ferrari & Chivers, 2006a; Ferrari, Vrtělová, Brown, & Chivers, 2012). Therefore, with only one instance of learning, it is possible, as potentially reflected in the increased variability in response to the four test odours, that a single training of multiple predators provides unreliable information about predators. Previous studies have been able to observe this by monitoring response to predator odours over multiple days. My results indicate, however, that although unobservable in the overall response to the odour, it may be possible to observe uncertainty more quickly by breaking the response period down into smaller time segments (i.e., within the 8-min response as opposed to observations over multiple days).

Chapter 3: Learning specificity for generalization of hybrid predator recognition: implications for knowledge of non-parental species

3.1 Introduction

In response to a constantly changing environment, studies have observed aquatic species that are able to effectively respond to novel situations and those that cannot. Multiple hypotheses have been proposed to account for the disparity in antipredator response between species, with a focus on behavioural and phenotypic plasticity—two adaptive mechanisms that appear to influence effective responses in novel environments (Sih, 2013). Specifically, Sih et al. (2010) emphasizes two hypotheses to explain the range of observed responses:

(1) The cue similarity hypothesis suggests that the ability for prey to respond to novel predators is based on the type of cue used and the degree of similarity between the known and unknown predator cues. More similar cues will facilitate response to novel predators whereas more dissimilar cues may increase prey susceptibility to attack.

(2) The prey naïveté hypothesis proposes that the evolutionary history between prey and a non-native predator may affect their ability to respond to novel species. The impact of the invasions or introductions can be substantial on evolutionary naïve prey, as prey may either not recognize novel predators as threats or not respond effectively or appropriately to these threats. Shared evolutionary history, however, is predicted to provide prey with appropriate responses to novel predators and the enhanced probability of recognizing these predators as threats.

The combination of prey naïveté and/or cue dissimilarity has the potential to result in higher predation and potential local extirpation in response to novel introduced or invasive species (Clavero & García-Berthou, 2005; Sih et al., 2010, 2011). In response, the mechanisms that facilitate prey responses to novel environments, and specifically to novel predators, have received significant attention over the past decade. Understanding these mechanisms will help researchers, biologists, and ecologists to comprehend and, hopefully predict, how ecosystems

and individual species may respond to climate change, habitat alteration, pollution, and other environmental changes.

One mechanism, generalization of predator recognition, has received increasing attention in hopes to further explore adaptive responses to novel predators. Generalization of predator recognition appears to be extended to predators that are closely-related to a known predator, perhaps due to more similar olfactory cues released, and greatly decreases as phylogenetic distance increases from a known predator. The observed patterns, however, do not thoroughly account for hybrid species. As a mating of two species, the characteristics of the resulting hybrid odour signature are still relatively unknown. Leaving much to be explore, an understanding of where hybrid species fit into this recognition continuum may help us to predict how prey will respond to these novel predator odours.

Chapter 2 showed the ability for fathead minnows to generalize predator recognition to a novel hybrid predator with knowledge of one or both parental trout species. It also illustrated the ability for prey to generalize from a known trout species to an unknown confamiliar species. From these results, I was curious to find out whether hybrid trout species share similar olfactory cues with other confamiliar trout species as well. If so, prey may not need specific knowledge of a parental trout species. Instead, it may be possible for prey to respond to novel hybrid trout with knowledge of other closely-related trout species; thereby increasing the bodies of water eligible to have hybrid trout stocked. Consequently, the purpose of my second experiment was focused on exploring whether fathead minnows could generalize their recognition from a non-parental salmonid species to a novel hybrid trout predator. This experiment was aimed to help me better understand the role of the parental odour on facilitating generalized recognition to novel, stocked hybrid predators. The design of this study was intended to investigate another facets of the hypothesis that phylogenetic distance between a novel predator and a known predator influences the intensity of generalized predator recognition. As previously predicted, the graded antipredator response would be reflected in minnow behaviour such that the greatest intensity of response would be towards the known predator with reduced antipredator intensity to each more distantly-related predator. A similar pattern would be observed in the time-series analysis, with a graded response from the known predator odour to the more distantly-related predator odours. As in Chapter 2, all odours will likely elicit some level of initial response during the early response period, but antipredator responses to the more distantly-related species' odours will be dropped

in the late response period. With knowledge of the non-parental species, response with slightly less intensity is predicted to the confamiliar parental species odour, as it has been demonstrated in previous studies (e.g., Chapter 2; Chivers et al., 2015; Ferrari, Gonzalo, Messier, & Chivers, 2007). Response to the hybrid trout is more uncertain to predict—if hybrid trout odour shares similar cue characteristics with other confamiliar species, I predict that response will be similar to the unknown parental species. Hybrid trout odour may be more dissimilar, however, potentially as a result of being produced from two trout species causing the odour to appear more distantly-related. If so, then generalization to the hybrid tiger trout would likely be minimal.

3.2 Methodology

3.2.1 Test Species – Collection and Maintenance

Fathead minnows and northern pike were collected and maintained as described in Chapter 2. The brook, tiger, and rainbow trout, however, were acquired from the Fort Qu'appelle Fish Culture Station (SK, Canada) for this experiment, kept in separate tanks, and fed a diet of trout pellets.

3.2.2 Stimulus Preparation

Alarm cues from fathead minnow were acquired following the protocol in Chapter 2. Following established protocol, predators were size-matched to standardize predator size and odour concentration (50 mL of clean dechlorinated water per 1 gram of fish) among species. Forty-eight h prior to stimulus collection, four size-matched brook trout (mean ± S.E. TL = 190.75 ± 4.5 mm), rainbow trout (mean ± S.E. TL = 191.3 ± 6.4 mm), tiger trout (mean ± S.E. TL = 189.3 ± 6.1 mm), and pike (mean ± S.E. TL = 213.5 ± 3.7 mm) were arbitrarily chosen and moved individually to identical 37 L tanks with clean dechlorinated water. All predators were starved for the 48 h to allow for maximum gastric evacuation before collection began (Bromley, 1994; He & Wurtsbaugh, 1993; Nilsson & Brönmark, 2000). Fish were then transferred into collection tanks (37 L) filled with a volume of clean dechlorinated water relative to the size of each fish (50 mL of water/g of fish). Predators were left for 24 h with no water replacement, removed and then fed once returned to original holding tanks. Predator-conditioned water was mixed, divided into 100 mL portions, and frozen (-20°C) until needed. All predators were housed in the same conditions (i.e., temperature, l:d cycle) as the minnows prior to and throughout the stimulus collection.

3.2.3 Experimental Protocol

My experiment followed a 2x4 design. Minnows were conditioned to learn either brook trout (BkPO) or rainbow trout (RnPO) as a predator. After 24 h, minnows were presented with one of the following predator odours: 1) BkPO, 2) TgPO, 3) RnPO, or 4) PiPO.

Conditionings were prepared and administered using the same technique as described in Chapter 2 (20 mL of PO followed by 5 mL of AC). Groups of 3 minnows were placed in conditioning tanks and acclimated for 48 h. Each tank was randomly assigned one of the two conditioning treatments. One hour after the final conditioning, minnows were moved to identical 37 L testing tanks with clean dechlorinated water and fed. Minnows were tested for recognition 24 h after the conditioning event, with injection of one of the test odours (as outlined above) into each tank. The number of lines crossed was recorded every 15 sec during the 8-min pre- and post-stimulus injection observation periods.

3.2.4 Statistical Analysis

Change in Overall Activity

Pre-stimulus baseline activity levels for treatment combinations were analyzed using a 2x4 ANOVA, with brook trout and rainbow trout as the two conditioning groups and (1) BkPO, (2) RnPO, (3) TgPO, and (4) PiPO as the test groups. As in Chapter 2, the number of lines crossed were computed to a change in line crosses (subtracting the number of lines crossed during the pre-stimulus observation period from the number of lines crossed during the post-stimulus observation period). The response variable data were then rank-transformed to account for heteroscedasticity and analyzed using a non-parametric 2-way ANOVA (Scheirer-Ray-Hare extension of the Kruskal-Wallis test, Sokal and Rohlf 2003). Significant interactions were analyzed by splitting the data into conditioning group (brook, rainbow), and then analyzed separately using a Kruskal-Wallis test (normal distribution of data but heterogeneous variances between tested stimuli). Significant differences were analysed with Games-Howell post hoc comparisons to investigate if change in line crosses differed among the injected predator odours.

Early versus Late Response

As in Chapter 2, we further investigated changes in activity over a time series. As the time series data were homoscedastic, the mean change in line crosses was analyzed using a 2x2x4 repeated measure ANOVA, with time (early, late) as the within-subjects variable and the

conditioning (RnPO, BkPO) and testing (BkPO, RnPO, TgPO, PiPO) odours as the between-subjects variables. Data were separated into conditioning groups, and differences in mean change in line crosses between the four test odours were compared within each time segment (early, late) using a 1-way ANOVA. Significant differences were analysed with Tukey HSD pairwise post hoc comparisons. The statistical analysis was carried out using IBM SPSS Statistics 22 with statistical significance set as $\alpha = 0.05$, unless otherwise noted.

3.3 Results

Change in Overall Activity

Pre-stimulus baseline activity levels were the same regardless of treatment group (test: $F_{3, 142} = 0.9$; condition: $F_{1, 142} = 0.3$; interaction: $F_{3, 142} = 0.6$), with an average of 13 lines crossed per minute. Change in line crosses was significantly affected by an interaction between conditioning odour (RnPO, BkPO) and testing odour (RnPO, BkPO, TgPO, PiPO) (non-parametric ANOVA: $H_{3, 142} = 41.5$, $p < 0.001$). When minnows learned to recognize brook trout as a predator, minnow behaviour was affected by the odour presented during testing (Kruskal-Wallis: $\chi^2 = 45.5$, df = 3, $p < 0.001$). Minnows responded similarly to the odours of the known predator, brook trout, and the hybrid predator, tiger trout ($p = 0.9$), and with a greater intensity than those exposed to the odour of the rainbow trout or pike control (all $p < 0.001$) (Fig. 3.1). Tested predator odours also elicited different responses in minnows conditioned to learn rainbow trout odour (Kruskal-Wallis: $\chi^2 = 39.4$, df = 3, $p < 0.001$); minnows responded most strongly to the known rainbow trout odour than to any other test odour presented (all $p < 0.001$) (Fig. 3.1).

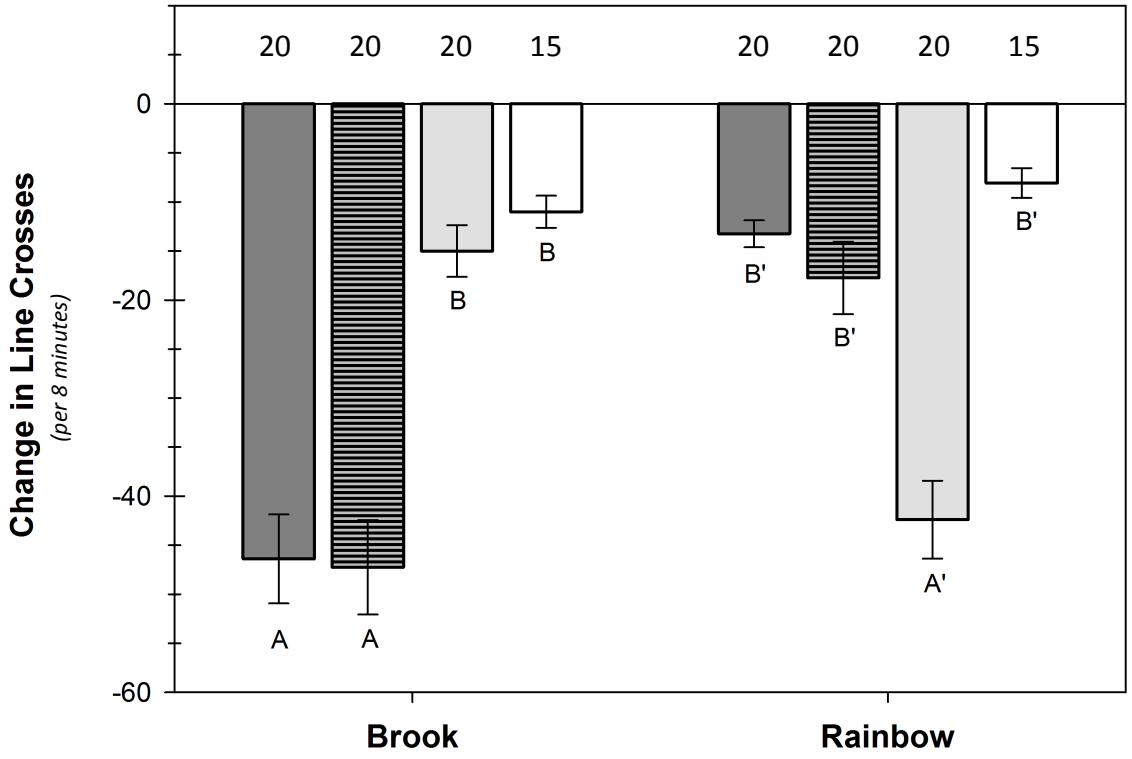

Figure 3.1: Mean (±S.E.) change in line crosses from pre-stimulus baseline for minnows conditioned to learn brook trout or rainbow trout as a predator then tested for recognition of brook trout (dark gray bars), tiger trout (gray stripped bars), rainbow trout (light gray bars) or northern pike (white bars) odour. The value above each bar indicates sample size. Letters indicate statistical significance (α = 0.05).

Early versus Late Response

Over the time series, change in line crosses were not affected by a three-way interaction among time (early, late), conditioning odour (BkPO, RnPO), and test odour (BkPO, RnPO, TgPO, PiPO) ($F_{3, 142} = 0.4$, $p = 0.8$); however, line crosses differed over time (time: $F_{1, 142} = 42.2$, $p < 0.001$) and were further affected by an interaction between conditioning and test odours ($F_{3, 142} = 39.9$, $p < 0.001$).

Minnows conditioned to learn brook trout as a predator responded differently to the four predator odours presented during the early response period ($F_{3, 71} = 11.1$, $p < 0.001$) and the late response period ($F_{3, 71} = 19.3$, $p < 0.001$). Initially, minnows exposed to the odour of the known predator, brook trout, responded with a significantly greater reduction in activity than those exposed to the rainbow or pike odours ($p = 0.01$), while the tiger trout odour elicited an intermediate response (see Fig. 3.2a for post hoc comparisons). Minnows exposed to brook trout and tiger trout odours maintained a similar high-intensity response into the late response period

(p = 0.9), while the response to rainbow trout odour was no different than the response to northern pike odour (p = 0.1).

The four test odours also elicited differences in line crosses for minnows trained to recognize rainbow trout as a predator during the early and late response periods (Early—$F_{3, 71}$ = 5.1, p = 0.003; Late—$F_{3, 71}$ = 12.7, p < 0.001). Minnows responded significantly more to the known rainbow trout odour than to the tiger trout and pike odours (p<0.04), while minnows tested with brook trout odour exhibited an intermediate response (Fig. 3.2b). Minnows tested with brook trout odour dropped their response during the late response period, leaving only minnows exposed to the known predator maintaining a significant antipredator response (Fig. 3.2b).

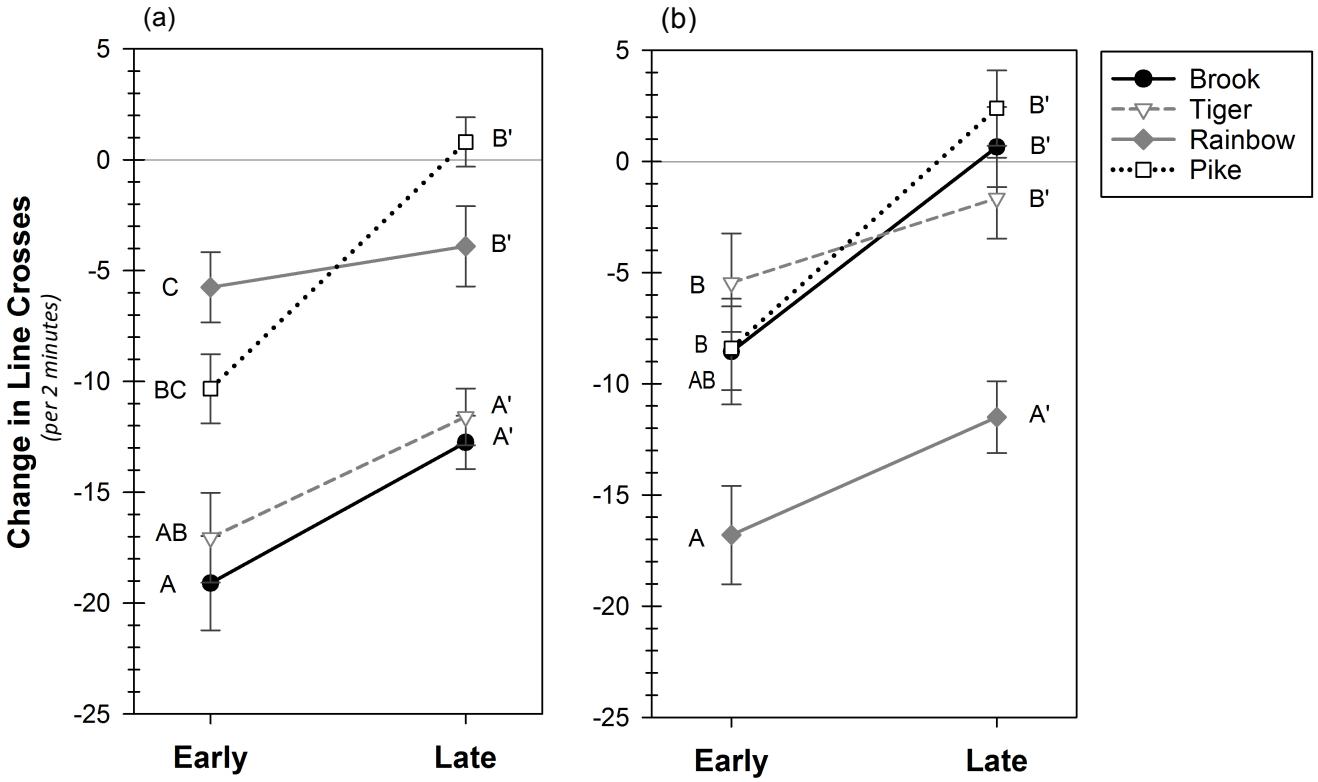

Figure 3.2: Mean (±S.E.) change in line crosses from pre-stimulus baseline during early and late response periods for minnows conditioned to recognize (a) brook trout or (b) rainbow trout as predators. Minnows from each conditioning group were then tested for recognition of brook trout (black lines, n = 20), rainbow trout (light gray lines, n = 20), tiger trout (gray dashes lines, n = 20), or northern pike (black dotted lines, n = 15) odour. Letters indicate statistical significance (α = 0.05).

3.4 Discussion

The data presented here indicates that knowledge of a parental species is necessary for generalized recognition of hybrid predators. Previous studies have demonstrated that generalized recognition of parental species from a known hybrid predator, as well as the reverse, is possible (Chivers et al., 2015; Chapter 2). My results, however, are the first to show that knowledge of closely-related species to the hybrids' parents may not be sufficient to elicit a generalized response to the odour of hybrid predators.

My results support previous findings about predator generalization, whereby known predator odours receive the strongest antipredator response during testing. With a 68% reduction in activity to brook trout odour following BkPO conditioning and rainbow trout odour following RnPO conditioning, my experiment demonstrates a conservation of response, whereby the intensity of antipredator response remains consistent when presented with the odour of a known predator. As minnows experienced similar background risk with only a single conditioning event, the parallel fear responses indicate that neither known trout odour is perceived as more dangerous than the other—conditioned trout odours are perceived as equally dangerous. Consistency in response may be reflective of a variety of factors, including but not limited to, the initial risk level associated with a known predator (e.g., Ferrari, Trowell, Brown, & Chivers, 2005; Helfman, 1989), the maximum level of innate response reflective of the risk posed by a known predator, and similarities in the extent of uncertainty surrounding the conditioned odour stimulus (Ferrari et al., 2010; Ferrari, Vrtělová, Brown, & Chivers, 2012).

My results further indicate that knowledge of a parental species is crucial for generalization to the hybrid tiger trout. When conditioned to learn brook trout as a predator, minnows displayed the predicted response pattern evident from previous studies: knowledge of a parental species facilitated generalization to the hybrid tiger trout with a response equal in intensity to that elicited by the known parental odour (Chapter 2). Surprisingly, minnows did not extend their generalization to confamiliar rainbow trout odour. Failure to generalize to confamiliar brook trout or the hybrid tiger trout odours was also displayed in minnows conditioned to learn rainbow trout as a predator. This contrasts with previous studies that showed generalization to the odour of confamiliar species (e.g., Chapter 2; Chivers et al., 2015; Ferrari, Gonzalo, Messier, & Chivers, 2007) but reflects the limit of generalization to only congeneric species demonstrated in other studies (e.g., Brown et al., 2011; Mitchell, McCormick, Chivers, &

Ferrari, 2013). One recent study brought to light the fact that generalization to predators even within the same family is not straightforward. Ferrari et al. (2016) found that highly certain prey, prey that had multiple experiences to learn a predator, generalized only to the most closely-related confamiliar predators. Conversely, prey with less certainty in their predator recognition, ones that had only a single training experience, generalized more widely to distantly-related species within the same family. These conflicting results indicate the great complexity surrounding the factors that affect generalization, and support the necessity to investigate how these differences arise.

Among species, there may be a variety of factors that influence the degree of generalization:

1) Amount or concentration of alarm cue, or unconditioned stimulus—Greater concentrations of alarm cue, indicative of higher risk, appear to enhance the generalization window to closely-related predators, whereas low risk seems to inhibit generalization to closely-related predators (Ferrari, Brown, Messier, & Chivers, 2009; Ferrari, Messier, & Chivers, 2008; Ferrari, Trowell, Brown, & Chivers, 2005);

2) Uncertainty surrounding the known predators—Increased number of conditioning, or learning, events have been shown to decrease uncertainty surrounding predator recognition, resulting in greater retention of the information (Ferrari et al., 2010; Ferrari et al., 2012). Decreased uncertainty has also been shown to help remedy conflicting information and influence the extent of generalization. Ferrari et al. (2016) demonstrated that high-certainty minnows generalized only to closely-related confamiliar species while low-certainty minnows generalized to more distantly-related confamiliar species. Likely, these additional learning events allowed the prey to refine their recognition of odour signatures within the known predator odour and only generalize to novel odours that exhibit the most comparable signatures.

3) Methodological differences—Test species are an obvious reason for differences found between studies. It has been suggested that species from different taxa acquire different amounts of information at various speeds from predators (Crane & Ferrari, 2013; Ferrari et al., 2009). This could, in turn, explain the differences in the extent of generalization observed between various species. Alternatively, differences in odour preparation, both unconditioned and conditioned stimuli, may impact results as well.

For instance, some studies create their predator odour from soaking the predators for 72 hours (e.g., Brown et al., 2011) or 24 hours (e.g., Ferrari et al., 2007) and then subsequently freeze the water and odour mixture, while others use fresh odours taken directly from tanks containing the predators (e.g., Mitchell et al., 2013). The differences here could impact the strength of the predator odour and thus could impact predator recognition and antipredator response intensity (Ferrari, Messier, & Chivers, 2006).

4) Predator size—Experienced minnows, for instance, can differentiate between large and small predator through odour recognition (Kusch, Mirza, & Chivers, 2004). Interestingly, the minnows responded most strongly to the odour of the smaller predators. It can be assumed that larger predators in the same volume of water would produce more odour than smaller predators. The stronger response to the smaller predators would not likely be a result of a higher concentration of predator odour. The results may instead reflect that the smaller predators are more ecologically relevant predators—the minnows used in that study were not naïve but had been acquired from a lake with a resident population of pike. Given their previous experience with pike as predators, it is likely that minnows had learned to differentiate ecologically relevant cues prior to collection. It remains unclear, however, what cues from the predator odours would have been used for the prey to differentiate size in the wild. Perhaps it has to do with physiological changes and thus different odour signatures being released throughout the predator's ontogeny. Further research should investigate the ability for prey to generalize given knowledge of younger or older predators.

5) Ambient risk associated with prey's natural environment—The 'generalization of predator recognition continuum' hypothesis suggests that the ability for prey to recognize predators would be greatly influenced by the predictability and diversity of predators present in an environment (Ferrari et al., 2007, 2008). This hypothesis predicts that learned predator recognition and generalization should be favoured in environments with a great variety of predators, as well as one in which the probability of being attacked is quite unpredictable. This strategy would require prey to continually update predator information and the associated risk levels during each

encounter, and then subsequently allow prey to response in a threat-sensitive manner to ecologically relevant threats. Generalization of predator recognition would further benefit these populations as they could use known predator characteristics in the form of a 'predator template' to identify potential predators that also exhibit similar characteristics. Prey that can plastically adapt their antipredator response to these novel, closely-related predators would then enhance their probability of survival by potentially eliminating the need for an initial learning encounter.

What factors, however, would influence minnows from the same population to generalize to confamiliar species in one instance and not in another? One possibility is that the methodology used to create the predator odour for Chapter 2 and 3 were quite different. The odour donated for Chapter 2 was prepared at Cold Lake Fish Hatchery (AB) using 40 fish from each species to create the predator odour, whereas there were four fish from each species acquired from the Fort Qu'appelle Fish Culture Station (SK) used to create the odour for Chapter 3. The predator odours were prepared with a consistent concentration—predators were size matched and then placed in a volume of water proportional to the size of the predators (50 mL/g of fish). Ideally, this results in similar concentrations of predator odour in the water; however, a few studies have demonstrated that even with the same overall concentration of predator odour, minnows can differentiate predator density, proximity, and relative size from the odour (Ferrari et al., 2006; Kusch, Mirza, & Chivers, 2004).

Kusch et al. (2004) found that the intensity of antipredator response in minnows increased with increasing concentration of predator odour, suggesting that minnows could determine either the proximity of the predator or the density of predators within a close-range. Ferrari et al. (2006) expanded on this idea to show that when minnows were exposed to the same volume of predator odour with the same overall concentration of predator size-to-water volume ratio, they could differentiate between odours created from 2 predators or 12 predators. Interestingly, the odour made from 2 predators was more threatening than the odour created from 12. These results indicate that the number of predators used in each of my experiment could drastically impact the minnows' responses. Higher threats have been shown to reduce the extent of generalization (Ferrari, Messier, & Chivers, 2008), and thus it is possible that the higher threat associated with only 4 predators would have inhibited generalization to the confamiliar rainbow trout odour in Chapter 3. In the same fashion, the larger number of predators used in the Chapter

2 odour may have facilitated enhanced generalization to the confamiliar brook or brown trout odour. Further investigation to observe the effects of predator density and proximity on the extent of generalization would help to clarify these conflicting interactions. Researchers studying generalization of predator recognition need to carefully consider the factors that influence the extent of generalization and work to further understand how generalization may shift in these different circumstances.

Chapter 4: General Discussion

Influences from both anthropogenic and natural processes have led to significant changes in the Earth's biomes. Compounded, these factors have generated shifts in the environment that may put a variety of species at elevated risk. Introduced and invasive species, for instance, can have a radical effect on resident populations, with the potential to drastically transform an entire ecosystem (Knapp et al., 2001). For prey, susceptibility to introductions or invasions of novel predators will heavily depend on their ability to respond to these novel predators. Generalization of predator recognition appears to help reduce this vulnerability. This adaptation provides prey with the tools necessary to respond to novel predators that shares similar characteristics to a known predator (see Brown et al., 2011; Davis et al., 2012; Ferrari et al., 2007; Griffin et al., 2001; Mitchell et al., 2013). My thesis explored this phenomenon in reference to the stocking of, and natural interaction with, hybrid trout species—specifically hybrid tiger trout that are preferentially stocked in Saskatchewan and Alberta lakes and streams. My results may have significant implications for conservation biologists, fisheries biologists and stocking agencies.

I first investigated the ability for minnows to generalize recognition to hybrid tiger trout odour from knowledge of one or both confamiliar parental species. My results revealed that generalization of predator recognition was possible to both the unknown hybrid tiger trout and the unknown confamiliar parental species. Comparable responses to all three trout may indicate that trout share relatively similar odours, possibly since feeding preferences, physiology and pheromones may be similar. Based on the results of Chapter 2, I performed a subsequent experiment to determine whether the observed response was consistent to all trout species and whether or not hybrid generalization was possible with knowledge of another confamiliar, yet non-parental species. In Chapter 3, my results revealed that specific knowledge of a parental species, as opposed to any confamiliar trout species, is important for strong generalization to the hybrid tiger trout. I found further support for our hypothesis that the extent of generalization of predator recognition would be influenced by the degree of phylogenetic similarity between the novel odours and the known predator(s). Inquisitively, researchers have observed varying degrees of generalization, even within the same prey species. Sometimes prey generalize their

response only to closely-related congeneric species, while other times they generalize out to confamiliar species. These results indicate the elevated complexity associated with the circumstances affecting generalization, supporting the notion that this adaptation is not fixed, but rather flexible and adaptive to changing situations. These studies are some of the first to begin exploring the extent of generalization to novel hybrid predators.

Interestingly, my results also reveal that the overall intensity of an antipredator response may not be able to tell the entire generalization story alone. In Chapter 2, analysis of behavioural changes during the total 8-min observation period indicated that there were no differences in response to the three trout odours (BkPO, BnPO, TgPO), regardless of which parental odour they initially learned to fear. Breaking down the response into early response and late response time segments, however, illuminated differences that were undetected by the larger analysis. Previous studies have indicated that certainty of predator recognition has played an important part in the duration for which a response is maintained (e.g., Chivers & Ferrari, 2013; Ferrari et al., 2010; Ferrari et al., 2012). These studies demonstrated that the degree of certainty did not initially affect the intensity of antipredator response the day after the learning events, as we observed in the overall analysis; however, increasing the number of conditionings, and thus increasing certainty, did lead to a higher intensity response maintained over the following week. My time series results demonstrate that generalization patterns varied even on the first day after conditioning events ceased, potentially reflecting the uncertainty that surrounded some of the novel odours. Threat-sensitive differentiation in response quickly reinforces that the cost associated with displaying antipredator behaviour in response to a non-threatening odour would be wasted energy. These results may allow studies investigating certainty and generalization to occur over a shorter timespan. Similarly, it may also have important implications for short-term recognition generalization studies—pointing out that small differences may be overlook if researchers do not analyze data across the response period.

4.1 Conservation Applications

My thesis explores the ability for prey to generalize their recognition from known predators to a novel hybrid predator. Hybrid fish have attracted the attention of anglers and become some of the most sought-after recreational fish species; as such, stocking programs for hybrid trout have become more common for stocking agencies across North America. Saskatchewan, in specific, stocks hybrid trout every odd year, introducing over 700,000 hybrids

in the last decade (Government of Saskatchewan, 2005-2015). With longstanding programs, like those in Saskatchewan, and new ones developing, like those in Alberta, my research may have significant impacts on overall assessment protocol prior to stocking new lakes and streams. My thesis illustrates that specific knowledge of a hybrid's parental species is essential to facilitate prey's initial recognition of these introduced species. Without knowledge of parental species, it may be detrimental to prey populations (both invertebrate and vertebrate) by reducing survivorship, abundance, and in extreme cases shifting the entire dynamic of the ecosystem (Allendorf et al., 2001; Eby et al., 2006; Kats & Ferrer, 2003; Knapp et al., 2001; Rahel, 2000).

One of the key challenges for Saskatchewan fisheries is "providing a diversity of fishing opportunities to meet the interests of distinct users where biologically and ecologically feasible" while also "managing species and size selective harvest to minimize negative impacts to fish communities" (Saskatchewan Ministry of Environment, 2013). Fish stocking is thus used as a management tool to help recover degrading populations, supplement existing populations, or create new fisheries for anglers; however there is growing recognition that this must be done in a way that reduces overall negative impacts. The results of my research, in combination with previous research on predator recognition and generalization, indicate that mitigation of these issues may be possible by understanding the predator recognition and evasion strategies of the resident species. Generalization of predator recognition, a plastic adaptive response to novel predators, can provide significant fitness advantages in the event of novel fish introductions or invasions (Gall & Mathis, 2010). For stocking, this means that calculated stocking of game fish in locations that have phylogenetically similar resident species could reduce the impacts of new introductions on prey species. Furthermore, stocking hybrids in aquatic systems that have previous experience with one or both of the parental species will help satisfy angler requests, while also reducing the probability that prey will be unable to respond. Finally, the stocking of tiger trout, a sterile hybrid, may help achieve provincial mandates to enhance new fisheries without having to worry about uncontrolled population growth; these sterile hybrids are easily manageable since they do not reproduce naturally. Enhanced understanding and intentional action-plans for stocking decisions will help to alleviate some of the harmful impacts associated with stocking.

Unexpectedly, knowledge of both parental species may inhibit the intensity with which prey respond to the novel hybrid tiger trout (Chapter 2). More research is certainly in order to

investigate the circumstances surrounding this phenomenon. If the observed change in response over time was a product of increased uncertainty, given only a single experience to learn each parental species (see Ferrari et al., 2012; Ferrari et al., 2016), increased experience with both parental species may actually enhance prey's ability to recognize and respond to novel hybrid predators. Conversely, if the observed trend is maintained, aquatic systems with both parental species of a hybrid fish may not be suitable locations for stocking of hybrid predators. This will create an additional factor for fisheries biologists to consider when assessing specific locations for hybrid introduction.

The movement and transport of non-native species often occurs as human-mediated events, either intentionally or unintentionally. Luckily, we can attempt to regulate species introduction by making well-informed stocking decisions and using public education to inform and spread awareness about the impacts of unintentional movement of species. On the contrary, there are many natural processes that we cannot control—processes that occur by chance such as during natural introgression, range expansion, or environmental changes (Rahel & Olden, 2008). So although we can manage where we stock hybrid fish, we cannot control where these hybrids may naturally occur. Sympatric populations of brook and brown trout, for instance, have been shown to interact reproductively in their natural environment (Cucherousset et al., 2008; Grant et al., 2002; Sorensen et al., 1995). Studies have shown that although the timing of spawning seasons for these species are slightly staggered, overlap occurs for a period of around four weeks in some instances (Cucherousset et al., 2008; Sorensen et al., 1995). Further shifts in spawning seasons may exacerbate this reproductive interference in currently sympatric populations. Other sympatric fish species have also have been shown to hybridize (see Chevassus, 1979; Loxterman, Keeley, & Njoroge, 2014; Todesco et al., 2016). It seems that natural hybridization is more common in fishes than in other vertebrate species (Allendorf et al., 2001), and the probability for natural hybridization is predicted to rise as previously separated habitats continue to overlap given warming aquatic conditions (Lynch et al., 2016). If subsequent research supports that prey cannot adequately recognize hybrid predators when both parental species are known, however, we could see a massive shift in food web dynamics in those environments. For conservation ecologists and biologists, it will be important to understand how this increase in naturally occurring hybrids may impact different aquatic systems.

Overall, the implications for understanding how prey generalize predator recognition will not only be important for conservation and stocking in regards to hybridized species, but also in terms of species invasion. Biological invasions have had significant ecological and economic impacts throughout the world, from irreversible damage to local ecosystems to reduction in bio-resources and recreational spaces (Sax et al., 2007). The projected effects of climate change, such as increased global and aquatic temperatures, precipitation patterns and increasing salinization, may greatly foster the ability for non-native species to expand into new habitats (Rahel & Olden, 2008). Detrimental invasions or introductions likely occur when the resident prey species have no previous evolutionary history with the novel predator or with any closely-related species; in these instances, the naïve prey hypothesis suggests that prey may be unable to effectively respond to the initial invasion or introduction of the novel predator (Sih et al., 2010). My results, as well as the plethora of literature on threat-sensitive generalization of predator recognition, however, indicate that this adaptation may provide prey with a survival advantage when responding to novel predators that are similar to previously known predators. Understanding if, and then how, various prey species can generalize their learned information may in fact illuminate how susceptible ecosystems may be to novel species invasions or introductions. Fishless aquatic systems are highly susceptible habitats to invasive fish (Caudill & Peckarsky, 2003; Knapp et al., 2001; Pister, 2001), whereas those with multiple fish predators and non-predators may fare comparatively better (Davis et al., 2012). Further research would allow us to understand how generalization may impact these ranges of ecosystems with varying degrees of fish community compositions. Although not all invasion events have negative consequences, the impact of various nuisance species has inspired a strong desire to mitigate these events. By increasing our knowledge of generalization of predator recognition, we can begin to take a more proactive prevention approach to mitigate the impacts of ecological invasions.

4.2 Future Directions

The ability for prey to recognize varying levels of threat and plastically respond in a threat-sensitive manner exemplifies a well-adapted predator avoidance mechanism; this adaptation has important implications for prey, allowing them to strategically allocate energy between antipredator behaviour and other fitness-related activities. Furthermore, prey species have been shown to use this information to generalize their response to novel predators on their

first encounter. Researchers have been investigating the extent of these predator recognition mechanisms to further understand how prey species are able to maximize their fitness in an ever-changing world. As discussed, the results of this research can be applied to fish stocking decisions and help to predict ecosystem susceptibility; however, there are many questions that need to be considered while we work towards effectively using this information to inform fisheries management decisions.

My research illuminated that prey can generalize to novel, hybrid predators when one or both parental species are known. We saw, however, that knowledge of both parental species actually lowered the strength with which the prey responded to the hybrid tiger trout odour. Further research is needed to understand what circumstances may have led to this response. Studies investigating increased knowledge of one or both parental species would help us to understand if the observed response was due to uncertainty surrounding previous knowledge. It could instead indicate that knowledge of both parental species may confuse prey when encountering novel hybrid predators. These results would have significant implications for conservation biologists understanding natural hybridization influences and for stocking agencies in deciding where to introduce novel hybrid trout.

Furthermore, studies should continue to investigate how the number of learning events, and thus certainty, may impact generalization to hybrid predators. This may help us understand how our results may be represented in nature. My results show how prey may respond if they have a single or double encounter with a known predator—perhaps chance encounters in a large habitat. This may be common in more open ecosystems, such as oceanic or steam habitats—and require a different response strategy. In freshwater ponds or lakes, however, the chance of encountering a predator living in the same area will likely be higher, and thus we may see different response strategies surface. A recent study by Ferrari et al. (2016) demonstrated that increasing certainty surrounding the known predator acted to widen and strengthen generalization to closely-related confamiliar predators, but narrowed the extent of generalization to more distantly-related confamiliar predators. With this in mind, it may be important to re-examine which confamiliar and congeneric fish species are used to understand generalization to hybrid predators. For the tiger trout specifically, I would be interested in taking Chapter 3 one-step further and investigating whether knowledge of lake trout (*Salvelinus namaycush*), a congeneric species to the paternal brook trout, could facilitate recognition of the hybrid tiger

trout. Additionally, studies altering the certainty of the prey will help us to understand if generalization to a hybrid predator is possible with increased knowledge of a non-parental trout species.

Natural variation in the extent of generalized predator recognition within a single prey species also became evident in my research. Fathead minnows conditioned in Chapter 2 with one or both parental species demonstrated generalized predator recognition to the hybrid trout and the novel, confamiliar parental species. The results of Chapter 3, however, did not reflect the same breadth of generalization as shown in Chapter 2—instead, minnows only extended generalized predator recognition from the known parental brook trout to the novel hybrid trout, but not to the novel confamiliar rainbow trout. When the known predator was the rainbow trout, generalized predator recognition was not extended to either the confamiliar brook trout or the hybrid tiger trout. One factor that may influence why this difference has occurred is the natural variation between minnows and their behavior. Although the minnows were acquired from the same pond, differences could be explained strictly by the natural variation that exists between subgroups of a population. Another factor proposed to explain these differences comes from varying methodology, specifically the difference in the age of predator odour. The predator odour used in Chapter 2 had been frozen for a year, while the predator odour from Chapter 3 was made two weeks prior to the experiment. It would be interesting to investigate whether predator odour, and alarm cue for that matter, degrades over a given period of time in a frozen state. If so, it could have important implications for future experiments and survival training of hatchery-reared fishes.

Variation in response has also been shown across aquatic taxa, with some species showing narrower generalization from a known predator to only congeneric species, while others have demonstrated wider generalization to confamiliar species. Thus far, the research that has been conducted on generalization of predator recognition has strongly focused on the hypothesis that knowledge of phylogenetically similar species will facilitate generalization from a known predator to an unknown predator—as phylogenetic distance increases, the ability to generalize from one predator to the next decreases. This hypothesis has received much support throughout the literature; however, in a natural environment, there are more factors that will affect a prey's ability to respond. Ecological similarity of predators affecting olfactory cues is one of them. Prey have been shown to recognize and respond to novel predators when conspecific alarm cue is

detected in their diet (e.g., Hill & Weissburg, 2014; Mirza & Chivers, 2003; Wisenden, 2000). Without alarm cue presence in the diet cue, other studies have found that diet cues, reflective of similar prey selection, do affect generalization abilities but are not alone responsible for facilitating generalization. In these instances, multiple sources of information were incorporated for the prey to refine their predator templates (Mitchell et al., 2015). Further research will benefit from incorporating a greater variety of information (e.g., auditory, visual, olfactory, mechanical, etc.) to understand how generalization of predator recognition may be exhibited in the wild. As generalization appears to be widespread among a variety of taxa but the extent of generalization appears to differ between species, over time, geographic location, and background risk, it will be important to continue to seek understanding into the proximate mechanisms influencing these predator recognition strategies.

One of the most important areas for future research will be investigating generalization of predator recognition in the natural aquatic environment. Thus far, generalized predator recognition using olfactory cues has been primarily investigated in the laboratory setting. Research seeking natural validation for the observations found in the laboratory will be crucial in determining the applicability of this research to ecological concerns. As mentioned, it will be important to understand how the combination of visual and olfactory cues influence a prey's ability to generalize predator recognition, as they will not always rely solely on chemical cues. It will also be imperative to understand if these generalized responses are effective to novel predators, such as hybrid trout. Although ecology of the closely-related predators may be similar, attack strategy and intensity may not. Hybrid tiger trout, for instance, have attracted substantial angler interest from across the Western provinces due to their aggressive nature, giving anglers unique and satisfying fishing experiences (Alberta Environment and Parks, 2015). If this behaviour is more intense than aggression exhibited by parental species, prey may be unable to adequately respond to the novel hybrids, regardless of their ability to generalize to them. Finally, other behavioural traits of these new hybrid species will be important to understand, such as reproductive success (for reproductively viable hybrids such as splake), reproductive interference, and competition with other resident predatory species. From a conservation standpoint, understanding how these behavioural traits influence native, and perhaps threatened, species will further inform our decisions on where hybrid fishes could sustainably be stocked.

With natural validation in mind, my findings could have implications for future conservation efforts in the light of climate change. Government of Canada has deemed climate change as "one of the greatest threats of our time" (Government Canada, 2016). Along with the warming trend observed in the Earth's atmosphere, subsequent changes have been reflected in our aquatic ecosystems: increased salinization, altered thermal profiles and stream flow patterns, overall warming of aquatic ecosystems, and reduced duration of ice cover on both freshwater and ocean bodies (Rahel & Olden, 2008). Consequently, shifts in species distribution and geographical ranges are becoming more prevalent—once restricted from certain habitats due to physiological constraints, warm- and temperate-water species are now able to move with the warming temperatures and persist in new geographic locations (see Smith et al., 2012). The geographic ranges of Arctic and coldwater species, however, are shrinking, minimizing the area in which they are able to survive and reproduce. These changing environments have already incurred significant amounts of stress on inland fish species, with immediate implications for coldwater species, species living in arid conditions, and many prey species (Lynch et al., 2016).

These abiotic and biotic changes epitomize how important conservation and fisheries management will continue to be in the coming years. With shrinking habitats and overlapping geographic ranges, work to maintain or resurrect native populations while enhancing recreational fisheries will remain at the forefront of Fisheries' concerns. Research investigating how ecosystems respond to climate change, the ensuing increase in natural hybridization, and how stocking will continue to impact the freshwater systems will be crucial to maintain the environmental, cultural, and economic benefits of our aquatic systems. This information will be helpful both for immediate usage but also for predicting potential changes to be observed in the future.

Literature Cited

Acquistapace, P., Hazlett, B. A., & Gherardi, F. (2003). Unsuccessful predation and learning of predator cues by crayfish. *Journal of Crustacean Biology*, *23*, 364–370.

Alberta Environment and Parks. (2015). Tigers in Alberta? Sounds Kind of Fishy!, https://albertaep.wordpress.com/2015/11/18/tigers-in-alberta-sounds-kind-of-fishy/.

Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution*, *16*, 613–622.

Berejikian, B. A., Tezaka, E. P., & LaRaeb, A. L. (2003). Innate and enhanced predator recognition in hatchery-reared Chinook salmon. *Environmental Biology of Fishes*, *67*, 241–251.

Blumstein, D. T., Daniel, J. C., Griffin, A. S., & Evans, C. S. (1999). Insular tammar wallabies (*Macropus eugenii*) respond to visual but not acoustic cues from predators. *Behavioral E*, *11*, 528–535.

Boguski, D. A., & Watkinson, D. A. (2013). Information in support of a recovery potential assessment of Mountain Sucker (*Catostomus platyrhynchus*), Milk River populations (Designatable Unit 2). *Fisheries and Oceans Canada, Freshwater Institute*, 23.

Bromley, P. J. (1994). The role of gastric evacuation experiments in quantifying the feeding rates of predatory fish. *Reviews in Fish Biology and Fisheries*, *4*, 36–66.

Brown, G. E. (2003). Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries*, *4*, 227–234.

Brown, G. E., Adrian, Jr, J. C., Patton, T., & Chivers, D. P. (2001). Fathead minnows learn to recognize predator odour when exposed to concentrations of artificial alarm pheromone below their behavioural-response threshold. *Canadian Journal of Zoology*, *79*, 2239-2245.

Brown, G. E., Bongiorno, T., DiCapua, D. M., Ivan, L. I., & Roh, E. (2006). Effects of group size on the threat-sensitive response to varying concentrations of chemical alarm cues by juvenile convict cichlids. *Canadian Journal of Zoology*, *84*, 1-8.

Brown, G. E., Ferrari, M. C. O., Malka, P. H., Russo, S., Tressider, M., & Chivers, D. P. (2011). Generalization of predators and nonpredators by juvenile rainbow trout: learning what is and is not a threat. *Animal Behaviour*, *81*, 1249–1256.

Brown, G. E., & Smith, R. J. F. (1998). Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery-reared fish to recognize chemical cues of a predator. *Canadian Journal of Fisheries and Aquatic Sciences*, *55*, 611–617.

Caudill, C. C., & Peckarsky, B. L. (2003). Lack of appropriate behavioral or developmental responses by mayfly larvae to trout predators. *Ecology*, *84*, 2133–2144.

Chevassus, B. (1979). Hybridization in salmonids: results and perspectives. *Aquaculture*, *17*, 113–128.

Chivers, D. P., Al-Batati, F., Brown, G. E., & Ferrari, M. C. O. (2013). The effect of turbidity on recognition and generalization of predators and non-predators in aquatic ecosystems. *Ecology and Evolution*, *3*, 268–277.

Chivers, D. P., & Ferrari, M. C. O. (2013). Tadpole antipredator responses change over time: what is the role of learning and generalization?. *Behavioral Ecology*, *24*, 1114–1121.

Chivers, D. P., Mathiron, A., Sloychuk, J. R., & Ferrari, M. C. O. (2015). Responses of tadpoles to hybrid predator odours: strong maternal signatures and the potential risk/response mismatch. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20150365.

Chivers, D. P., McCormick, M. I., Mitchell, M. D., Ramasamy, R. A., & Ferrari, M. C. O. (2014). Background level of risk determines how prey categorize predators and non-predators. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140355.

Chivers, D. P., & Smith, R. J. F. (1994a). Fathead minnows, *Pimephales promelas*, acquired predator recognition when alarm substance is associated with the sight of unfamiliar fish. *Animal Behaviour*, *48*, 597–605.

Chivers, D. P., & Smith, R. J. F. (1994b). The role of experience and chemical alarm signalling in predator recognition by fathead minnows, *Pimephales promelas*. *Journal of Fish Biology*, *44*, 273–285.

Chivers, D. P., & Smith, R. J. F. (1998). Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Ecoscience*, *48*, 338–352.

Clavero, M., & García-Berthou, E. (2005). Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution*, *20*, 110.

Crane, A. L., & Ferrari, M. C. O. (2013). Social learning of predation risk: a review and prospectus. In *In K. B. Clark (Ed.), Social learning theory: Phylogenetic considerations across animal, plant, and microbial taxa* (pp. 53–82). Hauppauge, NY: Nova Science.

Crane, A. L., & Ferrari, M. C. O. (2015). Minnows trust conspecifics more than themselves when faced with conflicting information about predation risk. *Animal Behaviour*, *100*, 184–190.

Cucherousset, J., Aymes, J. C., Poulet, N., Santoul, F., & Céréghino, R. (2008). Do native brown trout and non-native brook trout interact reproductively? *Naturwissenschaften*, *95*, 647–654.

Darwish, T. L., Mirza, R. S., Leduc, A. O. H. C., & Brown, G. E. (2005). Acquired recognition of novel predator odour cocktails by juvenile glowlight tetras. *Animal Behaviour*, *70*, 83–89.

David, M., Salignon, M., & Perrot-Minnot, M. J. (2014). Shaping the antipredator strategy: flexibility, consistency, and behavioral correlations under varying predation threat. *Behavioral Ecology*, *25*, 1148–1156.

Davis, D. R., Epp, K. J., & Gabor, C. R. (2012). Predator generalization decreases the effect of introduced predators in the San Marcos Salamander, *Eurycea nana*. *Ethology*, *118*, 1191–1197.

Dewitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, *13*, 77–81.

Dixson, D. L., Munday, P. L., & Jones, G. P. (2010). Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology Letters*, *13*, 68–75.

Eby, L. A., Roach, W. J., Crowder, L. B., & Stanford, J. A. (2006). Effects of stocking-up freshwater food webs. *Trends in Ecology and Evolution*, *21*, 576–584.

Fausch, K. D. (1988). Tests of competition between native and introduced salmonids in streams: what have we learned? *Canadian Journal of Fisheries and Aquatic Sciences*, *45*, 2238–2246.

Ferrari, M. C. O., Brown, G. E., Bortolotti, G. R., & Chivers, D. P. (2010). Linking predator risk and uncertainty to adaptive forgetting: a theorestical framework and empirical test using tadpoles. *Proceedings of the Royal Society B: Biological Sciences*, *277*, 2205–2210.

Ferrari, M. C. O., Brown, G. E., Jackson, C. D., Malka, P. H., & Chivers, D. P. (2010). Differential retention of predator recognition by juvenile rainbow trout. *Behaviour*, *147*, 1791–1802.

Ferrari, M. C. O., Brown, G. E., Messier, F., & Chivers, D. P. (2009). Threat-sensitive generalization of predator recognition by larval amphibians. *Behavioral Ecology and Sociobiology*, *63*, 1369–1375.

Ferrari, M. C. O., Capitania-Kwok, T., & Chivers, D. P. (2006). The role of learning in the acquisition of threat-sensitive responses to predator odours. *Behavioral Ecology and Sociobiology*, *60*, 522–527.

Ferrari, M. C. O., & Chivers, D. P. (2006a). Learning threat-sensitive predator avoidance: how do fathead minnows incorporate conflicting information? *Animal Behaviour*, *71*, 19–26.

Ferrari, M. C. O., & Chivers, D. P. (2006b). The role of latent inhibition in acquired predator recognition by fathead minnows. *Canadian Journal of Zoology*, *84*, 505–509.

Ferrari, M. C. O., & Chivers, D. P. (2009). Sophisticated early life lessons: threat-sensitive generalization of predator recognition by embryonic amphibians. *Behavioral Ecology*, *20*, 1295–1298.

Ferrari, M. C. O., Crane, A. L., & Chivers, D. P. (2016). Certainty and the cognitive ecology of generalization of predator recognition. *Animal Behaviour*, *111*, 207–211.

Ferrari, M. C. O., Gonzalo, A., Messier, F., & Chivers, D. P. (2007). Generalization of learned predator recognition: an experimental test and framework for future studies. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 1853–1859.

Ferrari, M. C. O., Messier, F., & Chivers, D. P. (2006). The nose knows: minnows determine predator proximity and density through detection of predator odours. *Animal Behaviour*, *72*, 927–932.

Ferrari, M. C. O., Messier, F., & Chivers, D. P. (2008). Can prey exhibit threat-sensitive generalization of predator recognition? Extending the Predator Recognition Continuum Hypothesis. *Proceedings of the Royal Society B: Biological Sciences*, *275*, 1811–1816.

Ferrari, M. C. O., Trowell, J. J., Brown, G. E., & Chivers, D. P. (2005). The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. *Animal Behaviour*, *70*, 777–784.

Ferrari, M. C. O., Vrtělová, J., Brown, G. E., & Chivers, D. P. (2012). Understanding the role of uncertainty on learning and retention of predator information. *Animal Cognition*, *15*, 807–813.

Ferrari, M. C. O., Wisenden, B. D., & Chivers, D. P. (2010). Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*, *88*, 698–724.

Gall, B. G., & Mathis, A. (2010). Innate predator recognition and the problem of introduced trout. *Ethology*, *116*, 47–58.

Gazdewich, K. J., & Chivers, D. P. (2002). Acquired predator recognition by fathead minnows: influence of habitat characteristics on survival. *Journal of Chemical Ecology*, *28*, 439–445.

Gerlai, R. (1993). Can paradise fish (*Macropodus opercularis*, Anabantidae) recognize a natural predator—an ethological analysis. *Ethology*, *94*, 127–136.

Ghirlanda, S., & Enquist, M. (2003). A century of generalization. *Animal Behaviour*, *66*, 15–36.

Government Canada. (2016). Canada's Way Forward on Climate Change. http://www.climatechange.gc.ca/default.asp?lang=En.

Government of Saskatchewan. (2005-2015). Stocked Waters Guides. *Environment Publication Centre*. http://www.publications.gov.sk.ca/deplist.cfm?d=66&c=4538.

Grant, G. C., Vondracek, B., & Sorensen, P. W. (2002). Spawning interactions between sympatric brown and brook trout may contribute to species replacement. *Transactions of the American Fisheries Society*, *131*, 569–576.

Griffin, A. S., Evans, C. S., & Blumstein, D. T. (2001). Learning specificity in acquired predator recognition. *Animal Behaviour*, *62*, 577–589.

Hazlett, B. A. (2003). Predator recognition and learned irrevelance in the crayfish *Orconectes virilis*. *Ethology*, *109*, 765–780.

He, E. E., & Wurtsbaugh, W. A. (1993). An empirical model of gastric evacuation rates for fish and an analysis of digestion in piscivorous brown trout. *Transactions of the American Fisheries Society*, *122*, 717–730.

Helfman, G. S. (1989). Threat-sensitive predator avoidance in damsel-trumpet fish interactions. *Behavioural Ecology and Sociobiology*, *24*, 47–58.

Hill, J. M., & Weissburg, M. J. (2014). Crabs interpret the threat of predator body size and biomass via cue concentration and diet. *Animal Behaviour*, *92*, 117–123.

Holmes, T. H., & McCormick, M. I. (2010). Smell, learn and live: The role of chemical alarm cues in predator learning during early life history in a marine fish. *Behavioural Processes*, *83*, 299–305.

Kats, L. B., & Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience*, *5*, 361–394.

Kats, L. B., & Ferrer, R. P. (2003). Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions*, *9*, 99–110.

Kattenfeld, M., & Abrahams, M. (1997). The role of turbidity as a constraint on predatory-prey interactions in aquatic environments. *Behavioural Ecology and Sociobiology*, *40*, 169–174.

Kelley, J. L., & Magurran, A. E. (2003). Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries*, *4*, 216–226.

Knapp, R. A., Corn, P. S., & Schindler, D. E. (2001). The introduction of nonnative fish into wilderness lakes: good intentions, conflicting mandates, and unintended consequences. *Ecosystems*, *4*, 216–225.

Kusch, R. C., Mirza, R. S., & Chivers, D. P. (2004). Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. *Behavioral Ecology and Sociobiology*, *55*, 551–555.

Langerhans, R. B., & DeWitt, T. J. (2002). Plastically constrained: over-generalized induction cues cause maladaptive phenotypes. *Evolutionary Ecology Research*, *4*, 857–870.

Lima, S. L., & Bednekoff, P. A. (1999). Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *The American Naturalist*, *153*, 649–659.

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, *68*, 619–640.

Lönnstedt, O. M., & McCormick, M. I. (2011). Chemical alarm cues inform prey of predation threat: The importance of ontogeny and concentration in a coral reef fish. *Animal Behaviour*, *82*, 213–218.

Losos, J. B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*, *11*, 995–1003.

Loxterman, J. L., Keeley, E. R., & Njoroge, Z. M. (2014). Evaluating the influence of stocking history and barriers to movement on the spatial extent of hybridization between westslope cutthroat trout and rainbow trout. *Canadian Journal of Fisheries and Aquatic Sciences*, *71*, 1050–1058.

Lynch, A. J., Myers, B. J. E., Chu, C., Eby, L. A., Falke, J. A., Kovach, R. P., Krabbenhoft, T. J., Kwak, T. J., Lyons, J., Paukert, C. P., & Whitney, J. E. (2016). Climate change effects on North American inland fish populations and assemblages. *Fisheries*, *41*, 346–361.

Manassa, R. P., Dixson, D. L., McCormick, M. I., & Chivers, D. P. (2013). Coral reef fish incorporate multiple sources of visual and chemical information to mediate predation risk. *Animal Behaviour*, *86*, 717–722.

Mathis, A., & Smith, R. J. F. (1993). Fathead minnow, *Pimephales promelas*, learn to recognize northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike's diet. *Animal Behaviour*, *46*, 645–656.

Mathuru, A. S., Kibat, C., Cheong, W. F., Shui, G., Wenk, M. R., Friedrich, R. W., & Jesuthasan, S. (2012). Chondroitin fragments are odorants that trigger fear behavior in fish. *Current Biology*, *22*, 538–544.

Mery, F., & Kawecki, T. J. (2016). A fitness cost of learning ability in Drosophila melanogaster. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 2465–2469.

Mineka, S., & Cook, M. (1986). Immunization against the observational conditioning of snake fear in rhesus monkeys. *Journal of Abnormal Psychology*, *95*, 307–318.

Minto, C., Myers, R. a, & Blanchard, W. (2008). Survival variability and population density in fish populations. *Nature*, *452*, 344–347.

Mirza, R. S., & Chivers, D. P. (2000). Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. *Canadian Journal of Zoology*, *78*, 2198–2208.

Mirza, R. S., & Chivers, D. P. (2001). Chemical alarm signals enhanve survival of brook charr (*Salvelinus fontinalis*) during encounters with predatory chain pickerel (*Esox niger*). *Ethology*, *107*, 989–1005.

Mirza, R. S., & Chivers, D. P. (2003). Predator diet cues and the assessment of predation risk by juvenile brook charr: do diet cues enhance survival? *Canadian Journal of Zoology*, *81*, 126–132.

Mirza, R. S., Ferrari, M. C. O., Kiesecker, J. M., & Chivers, D. P. (2006). Responses of American toad tadpoles to predation cues: behavioural response thresholds, threat-sensitivity and acquired predation recogntion. *Behaviour*, *143*, 887–889.

Mitchell, M. D., Chivers, D. P., McCormick, M. I., & Ferrari, M. C. O. (2015). Learning to distinguish between predators and non-predators: understanding the critical role of diet cues and predator odours in generalisation. *Scientific Reports*, *5*, 13918.

Mitchell, M. D., McCormick, M. I., Chivers, D. P., & Ferrari, M. C. O. (2013). Generalization of learned predator recognition in coral reef ecosystems: how cautious are damselfish? *Functional Ecology*, *27*, 299–304.

Mitchell, M. D., McCormick, M. I., Ferrari, M. C. O., & Chivers, D. P. (2011). Friend or foe? The role of latent inhibition in predator and non-predator labelling by coral reef fishes. *Animal Cognition*, *14*, 707–714.

Murray, D. L., Roth, J. D., & Wirsing, A. J. (2004). Predation risk avoidance by terrestrial amphibians: the role of prey experience and vulnerability to native and exotic predators. *Ethology*, *110*, 635–647.

Nilsson, P. A., & Brönmark, C. (2000). The role of gastric evacuation rate in handling time of equal-mass rations of different prey sizes in northern pike. *Journal of Fish Biology*, *57*, 516–524.

Pister, E. P. (2001). Wilderness fish stocking: history and perspective. *Ecosystems*, *4*, 279–286.

Rahel, F. J. (2000). Homogenization of fish faunas across the United States. *Science (New York, N.Y.)*, *288*, 854–856.

Rahel, F. J., & Olden, J. D. (2008). Assessing the effects of climate change on aquatic invasive species. *Conservation Biology*, *22*, 521–533.

Saskatchewan Ministry of Environment. (2013). Fisheries Management Plan 2013-2018.

Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., Grosberg, R. K., Hastings, A., Holt, R. D., Mayfield, M. M., O'Connor, M. I., & Rice, W. R. (2007). Ecological and evolutionary insights from species invasions. *TRENDS in Ecology and Evolution*, *22*, 465–471.

Sih, A. (2013). Understanding variation in behavioural responses to human-induced rapid environmental change: A conceptual overview. *Animal Behaviour*, *85*, 1077–1088.

Sih, A., Bolnick, D. I., Luttbeg, B., Orrock, J. L., Peacor, S. D., Pintor, L. M., Preisser, E., Rehage, J. S., & Vonesh, J. R. (2010). Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos*, *119*, 610–621.

Sih, A., Ferrari, M. C. O., & Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications*, *4*, 367–387.

Smith, A. L., Hewitt, N., Klenk, N., Bazely, D. R., Yan, N., Wood, S., Henriques, I., MacLellan, J. I., & Lipsig-Mumme, C. (2012). Effects of climate change on the distribution of invasive alien species in Canada: a knowledge synthesis of range change projections in a warming world. *Environmental Reviews*, *20*, 1–16.

Sokal, R. R. and F. J. Rohlf. (1995). Biometry: the principles and practice of statistics in biological research, 3rd ed. New York: W.H. Freeman.

Sorensen, P. W., Cardwell, J. R., Essington, T., & Weigel, D. E. (1995). Reproductive interactions between sympatric brook and brown trout in a small Minnesota stream. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*, 1958–1965.

Stankowich, T., & Coss, R. G. (2007). The re-emergence of felid camouflage with the decay of predator recognition in deer under relaxed selection. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 175–182.

Todesco, M., Pascual, M. A., Owens, G. L., Ostevik, K. L., Moyers, B. T., Hübner, S., Heredia, S. M., Hahn, M. A., Caseys, C., Bock, D. G., & Rieseberg, L. H. (2016). Hybridization and extinction. *Evolutionary Applications*, *9*, 892–908.

Webb, J. K., Du, W. G., Pike, D. A., & Shine, R. (2009). Chemical cues from both dangerous and nondangerous snakes elicit antipredator behaviours from a nocturnal lizard. *Animal Behaviour*, *77*, 1471–1478.

Webb, J. K., Du, W., Pike, D., & Shine, R. (2010). Generalization of predator recognition: Velvet geckos display anti-predator behaviours in response to chemicals from non-dangerous elapid snakes. *Current Zoology*, *56*, 337–342.

Wisenden, B. D. (2000). Olfactory assessment of predation risk in the aquatic environment. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *355*, 1205–1208.

Wisenden, B. D. (2003). Chemically mediated strategies to counter predation. In S. P. Collin & N. J. Marshall (Eds.), *Sensory Processing in Aquatic Environments* (pp. 236–251). New York, NY: Springer-Verlag.

Wisenden, B. D., & Millard, M. C. (2001). Aquatic flatworms use chemical cues from injured conspecifics to assess predation risk and to associate risk with novel cue. *Animal Behaviour*, *62*, 761–766.

Wisenden, B. D., Vollbrecht, K. A., & Brown, J. L. (2004). Is there a fish alarm cue? Affirming evidence from a wild study. *Animal Behaviour*, *67*, 59–67.

Woody, D. R., & Mathis, A. (1998). Acquired recognition of chemical stimuli from an unfamiliar predator: associative learning by adult newts, Notophthalmus viridescens. *Copeia*, *4*, 1027–1031.

Zhao, X., Ferrari, M. C. O., & Chivers, D. P. (2006). Threat-sensitive learning of predator odours by a prey fish. *Behaviour*, *143*, 1103-1121.